AN INVESTIGATION INTO

A YEAST-LIKE FUNGUS

ISOLATED FROM PATIENTS SUFFERING FROM, OR SUSPECTED OF, PULMONARY TUBERCULOSIS

BY

EINAR HOLLSTRÖM

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ALMQVIST & WIKSELLS BOKTRYCKERI A.-B.

AN INVESTIGATION INTO
A YEAST-LIKE FUNGUS
ISOLATED FROM PATIENTS SUFFERING FROM, OR SUSPECTED OF, PULMONARY TUBERCULOSIS

Inaugural Dissertation

BY

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FROM THE INSTITUTE OF HYGIENE AND BACTERIOLOGY AT THE
UNIVERSITY OF UPSALA, SWEDEN.
CHIEF: PROFESSOR JOHN REENSTIERNA, M. D.

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Preface.

This investigation has been carried out at the Institute of Hygiene and Bacteriology at the University of Upsala. I now wish to express my sincere gratitude to its Chief, Professor John Reenstierna, for his having aroused my interest in the sphere of research within which my work lies.

I also wish to thank Dr. Nils Gellerstedt for the great trouble he has taken to examine my entire material of sections; Professor Fredrik Berg for his kindness in examining the ocular changes that had occurred in the test animals; Dr. Erik Hedvall for his assistance in examining the X-ray films; and Professor Robin Fåhræus and Professor Torsten Teorell for their kindness in placing at my disposal photographic equipment and micromanipulator.

I also beg to thank the assistants at the Institute, especially Dr. Vilhelm Hallberg, for their help and advice; Dr. Arne Franzell for assistance in taking the photographs; Miss Elli Öhrvall for her meticulously carried out drawings, and Mr. William Savage for his translation into English.

Last but not least I wish to convey my thanks to the Swedish Anti-Tuberculosis League for defraying the bulk of the expenses of my research work, and to the Regnell Fund of the University of Upsala for its contribution towards expenses.

Upsala, in April 1943.

Einar Hollström.

Introduction.

The class *Eumycetes* or true fungi has been subdivided into minor groups, according to their methods of sexual reproduction, but there is one large group, the *Fungi Imperfecti*, about which no method of sexual reproduction has been demonstrated. It is to this group that most of the pathogenic fungi important in medicine, belong. Many attempts have been made to classify these fungi, partly through their growth under certain conditions, partly through their capacity to disintegrate carbohydrates, by their serological qualities, and by their pathogenity in animal inoculation. But as yet no satisfactory solution of this problem has been found. The chief reason for this is the extreme phleomorphism in the fungi in question, especially in cultures. The indefinite classification has entailed confusion in taxonomy. As regards tests at classification and taxonomy of the various yeast-like fungi, reference is made to monographs and major treatises on the subject in question, e. g. Lafar (64), Vuillemin (134), Castellani and Chalmers (23), Sartory (107), Benedek (8), and Conant (26).

Yeast-like fungi have by many authors been demonstrated in the human body, particularly in morbid conditions, and they have by many been shown to be capable of being the cause of such. It is of especial interest to note that a yeast-like fungus has been pointed out as the mother-organism of Koch's bacillus. This opinion, which was advanced in 1912 by Reenstierna (98, 103), was based partly upon findings of such fungus in different tuberculous material, partly upon his observations that the fungus in question (single cell culture) was able to produce acid-fast rods. Further investigations in this matter have been carried out by Gullberg (41) in 1933—1935, but these were broken off by his death. My interest in this sphere of research was then aroused and was further incited when, in the year 1935, I could establish in a tubercle bacillus culture the appearance of fungus cells some of which contained short acid-fast rods.

As our knowledge of yeast-like fungi, especially their capacity to produce acid-fast rods, must as yet be looked upon as rather circumscribed and limited, an investigation into the subject seemed to me as likely of being of some value.

The initial material for my research consisted of five strains of fungus of yeast-type, isolated from patients either suffering from pulmonary tuberculosis or under observation with a suspicion of such.

In the investigation I have wished to study in the fungus strains:

a) some of their morphological, tinctorial, and biochemical properties, as well as their ability to produce acid-fast rods in culture;
b) their pathogenity in intravenous inoculation into rabbits and their ability to produce acid-fast rods in the animal organism; and
c) to investigate the pathogenity of the acid-fast rods obtained.

CHAPTER I.

Historical Survey.

Yeast-like fungi of various species occur very commonly in nature. As regards their occurrence in a healthy human being, opinions differ. Thus Todd (126) by way of example states that in 1937 he found in the mouth or throat of 1.000 healthy persons a fungus of yeast-type in 14.7 per cent. and Knighton (55) that in 1939 he had been able to demonstrate a similar fungus in 36.9 per cent of the oral cavities examined. Pijper (91), on the other hand, declares in a work in 1923, first by reason of his own experience, and second as the result of investigations in 1922, by Küster (63) and Bloomfield (14) as well as Wall (128) in 1923, that yeast-like fungus is no common micro-organism in the mouth and throat of healthy human beings. Even in other parts of the human body such fungus has been mentioned as normally occurring, in the ventricle and also in the whole intestinal region, in the skin, in the lachrymal fluid, etc.

A remarkably large literature exists about the occurrence of yeast-like fungus in morbid conditions, when the fungus has been looked upon either as a cause of disease or as a secondary invader. As is well known, Langenbeck (66), discovered in 1839 in thrush such a fungus, which Berg (10) a few years later proved to be the causative agent of this affection.

As is to be found in a survey by Plaut and Grütz (95), also animal experiments have been carried out, by several investigators, with fungus strains isolated from thrush, probably not belonging to one and the same species. — Cf. Tanner's and Dack's statement (125). — Regarding experiments with intravenous inoculations into rabbits, the following may especially be mentioned: 1896 Ostrowsky (87) could establish a rise in temperature (up to 41.2° C) with ensuing hypothermy (down to 33° C) which still existed at the animals death within 7 days; 1897 Steiner (117) observed, besides mycotic changes of the pectoral and abdominal organs, even such in

the central nervous system; Buss (16) in 1913 showed that the fungus contained endotoxins and was able to produce ectotoxins; Krauspe (59) in 1927 also proved the existence of endotoxins and found that these made the animals cachectic. Although not stated that the yeast-like fungus used in his experiments (intravenous inoculations into rabbits) originated from thrush, the interesting establishment by Stock (119), in 1908, of the appearance of ocular changes in the test animals should be mentioned in this connection. The ocular changes were: Conjunctivitis folliculosa, focal iritis, cyclitis and chorioiditis disseminata, focal affections in the retina, with delimited retinal loosening (clinically the affection was reminiscent of retinitis proliferans), as well as perineuritis nervi optici.

As to other morbid conditions in which the occurrence of yeast-like fungi have been established — vide some recently published surveys by Mitchell (81), Baker and Brian (5), Benedek (8), Sjövall (115) — the following should here be mentioned: ulcus ventriculi, where in 1922 Frank (37) and Cafasso (17) found such a fungus in more than 50 per cent; faeces in tropical sprue, stated by Kohlbrügge (56) in 1901, by Anderson (1) in 1917, and by Ashford (3) in 1929; vaginal affections, skin diseases, heart lesions, and the central nervous system; corneal affections, demonstrated in 1898 by Stöwer (124) and in 1900 by Lundsgaard (71); lower eyelid (tuberculoid changes but without tubercle bacilli), described in 1928 by Ferguson (33).

With regard to *lung affections*, numerous findings of various species of yeast-type fungus have been reported. Thus for example Stowall and Bubolz (121) state in 1929 that from the sputum of 100 patients with pulmonary conditions they had isolated 40 strains of yeast-like fungus forms. Many investigators have considered the fungus as the causative agent of the disease: In 1898 Artault (2); 1905 Castellani (18); 1913 Forgues (36); 1914 Mautner (76); 1915 Boggs and Pincoffs (15); 1917 Pijper (90) and Simon (114); 1919 Magrou (74); 1920 Castellani, Douglas, and Thomson (24), Chalmers and Macdonald (25), Jacono (49), and Nathan (86); 1921 Mendelson (79); 1922 Sartory and Moinson (108), Servet (113); 1923 Farah (31), Joekes and Simpson (50), Pijper (91); 1926 Nasso (85), Kotkis, Washowiak, and Fleisher (58); 1927 Wallace and Tanner (129); 1928 Galbreath and Weiss (39), Stowall and Greely (123); 1929 Grossi and Balog (40), Kurotchkin and Chu (62); 1930 Panayotatou (88), Stokes, Kiser, and Smith (120);

1931 Warr (130); 1933 Stowall and Bubolz (122), and Bergman, Danielsson, Ottander, and Schnell (11); 1934 Bakst, Hazard, and Foley (6); 1935 J., R., and Z. Flinn (35); 1937 Black and Eddy (13), Davis and Warren (29), R. and J. Flinn (34), Spreng (116), and others whose papers I have not had an opportunity to study.

Castellani's works (18, 19) of the years 1905 and 1910 have been looked upon as the first contributions of any importance to our knowledge of bronchial mycosis caused by yeast-like fungus. He elucidated there that the disease which was common amongst the tea-workers in Ceylon, and passed under the term of "teatasters' cough" and "tea-factory cough", was caused by a fungus, generally of yeast-type. Since then a large literature on pulmonary affections, which have been considered as being caused by such a fungus, has been published, and especially in recent years it has increased greatly but without anything like uniformity in the taxonomy. In the literature in the English language the term broncho-moniliasis has generally been used, inasmuch as the cause of the affection had been considered either the Genus Monilia, according to Castellani's and Chalmers' Table[1] (23) or according to Benham's (9) expression "monilia in a medical sense", i. e. all yeast-like, filamentous fungi. The term Blastomyces has been used especially by American pathologists to express a budding fungus of yeast-type. A good illustration of the deplorable confusion is Vuillemin's statement: "Il existe bien un genre Blastomyces, mais, quelque étrange que cette assertion paraisse, les Blastomyces ne sont pas de Blastomycètes."

So-called primary broncho-moniliasis has by Castellani been divided into three degrees of gravity, viz. mild, intermediate, and grave, and has in the main been described in the following way: In the mild type the patient's general condition is unaffected and there is no fever. The trouble consists of a slight cough with mucopurulent expectoration, often scanty and only in exceptional cases tinged with blood. With physical investigation at the outside the ordinary signs of bronchitis are found. The intermediate type is characterized by a slight bronchitis and slight fever of long duration. The more or less troublesome cough is frequently of a paroxysmal character, occurring in the mornings and at night, sometimes also moderate dyspnoea. In the grave form the symptoms, the same as the entire

[1] The definition of the Genus Monilia is, according to Castellani's and Chalmers' own statement, vague.

clinical picture, and many times also the X-ray picture, are in full agreement with pulmonary tuberculosis. The patient has acute fever attacks, progressive emaciation, night sweats, pains in the chest, troublesome cough, and sometimes dyspnoea, as well as repeated haemoptyses. The sputum is mucopurulent, of varying quantity, but mostly considerable. Anaemia exists frequently to a fairly high degree, occasionally also eosinophilia and some time an increase in the white corpuscles. With physical examination the same findings can be obtained as in advanced pulmonary tuberculosis. The X-ray picture may show pleural congestions, enlarged hilus shades, large cloudy infiltrations, generally in the lower parts of the lungs, but some times also in the apices. Cavities are also occasionally met with. The different symptoms and findings by investigation may vary to a high degree, and a primary diagnosis of fungal infection in the lungs cannot be made at the bedside.

Even the pulmonary affections in those cases where the causative agent by the respective author has been classified as a blastomycete, have shown great likeness to tuberculosis, which is apparent, by way of example, from the following statement by Servet in 1922 (113): "Cliniquement, rien ne ressemble plus à la tuberculose que les blastomycoses pulmonaires."

A few accounts of autopsical findings in cases with pure bronchomoniliasis, thus without the occurrence of other micro-organisms which may be looked upon as causative agents, have been published, viz. in 1915 by Boggs and Pincoffs (15); 1921 by Mendelson (79); 1931 by Warr (130) and in 1937 by R. and J. Flinn (34). In the case related by Boggs and Pincoffs there was present also a cancer mammae sinister with abscesses, which communicated with the cavity of the chest. These authors give a description which agrees with that for an acutely or subacutely pneumonized lung. A similar picture seems to have been present in R. and J. Flinn's case, pneumonic areas in both upper lobes and left pleural effusion. — They describe furthermore in the brain yeast colonies on the surface. — Mendelson's case showed in the affected upper lobe small "tubercles", which in reality were mycotic tumours without necrosis, and stand out as very prominent masses. In the interesting case described by Warr the left lung especially was adherent to the thorax, displaying a severely congested pleura visceralis. — The patient had previously suffered from pleuritis. — The lung showed a diminution

in volume to about one-third the normal size, and was of firm consistency. It was pervaded by cavities, the largest about 4 cm in diameter, and each connected with a bronchus. These gave the lung a cheese-like appearance. Most of the cavities were smoothly lined and many were filled with a clear, jelly-like material. There was but little air-bearing in either lobe. In the right lung existed analogous changes, though less pronounced. The microscopical picture showed nothing characteristic. In a survey-work in 1927, with the title "Notes on Certain Bronchomycoses Which may Simulate Pulmonary Tuberculosis", Castellani (21) points out in pulmonary blastomycosis[1] the anatomo-pathological similarity to tuberculosis. The lungs may be seen to be studded with numerous white nodules. The cut-surface may show a purulent fluid, and cheesy material may be expressed from the involved region. Cavities of various sizes may be present. Histologically, central necrotic areas are seen surrounded by a zone of granulation tissue, in which giant cells are found, some of which seem to contain blastomyces.

The diagnosis of broncho-moniliasis is based on findings of yeast-like fungus not only in smears from the sputum but also by culture. Moreover, it is required that the fungus should be present in fairly large quantities, and increase in numbers with the progress of the disease. The isolation of fungi from sputum has as a rule been done by spreading in plates of a sugary medium. The simplest method seems to me, however, to be the one mentioned by Pijper (91) in 1923, who made use of the tolerance in yeast-like fungi of such a high degree of acidity, that bacteria do not endure it. He transferred the sputum from cases of broncho-moniliasis to media with ph 2, where the fungus grew, but not the bacteria existing in the sputum.

With regard to the diagnosis of broncho-moniliasis, it is most important to be able to exclude other pulmonary affections, and then particularly tuberculosis. In order to facilitate the diagnosis, Castellani has recommended intra-pulmonary inoculations into rab-

[1] In Castellani's paper the Blastomyces' are characterized in the following manner: "In the lesions large round or oval cells are present, with an exceedingly well-marked double contour and with protoplasm very granular. No mycelium occurs in the lesions. Cultures may present a fluffy appearance (due to aereal hyphae) and contain a large amount of mycelium. Asci have been described by certain authors. They do not produce gas fermentation of sugars."

bits of the isolated fungus. Tests have been carried out with complement fixation and agglutination reaction, using serum and fungal strains isolated from the cases in question. Farah (31) states in 1923 that he had succeeded in obtaining positive agglutination reaction, while in 1929 Grossi and Balog (40), as well as Hoffstadt and Lingenfelter (47), noted that the fungus cells showed spontaneous agglutination. Complement deviation was obtained in 1923 by Farah (31) and Steinfield (118), and in 1929 by Hoffstadt and Lingenfelter (47), and also in the same year by Kurotchkin and Chu (62). Negative results have, however. according to several authors, been stated. Tests for using skin reactions as an aid in diagnostics have also been made with varying success, and in regard to this subject the reader is referred to Balog and Grossi's (7) investigations in 1929.

As regards the prognosis of broncho-moniliasis, the mild type may result in a spontaneous cure, while the intermediate type may heal spontaneously after a long time, although often with exacerbations, or pass into the grave form, which terminates fatally. The ordinary therapy, which according to statement seems to have had the greatest effect upon not too severe cases, is potassium iodide in large doses. Furthermore, autovaccin is also by some authors said to have excercised a certain amount of influence, but this is denied by many.

As has been stated in the preceding, tuberculosis has been most important as being capable of exclusion in diagnosing broncho-moniliasis. In several instances yeast-like fungus has been met with in sputum together with tubercle bacilli: In 1920 Castellani. Douglas, and Thomson (24); 1921 Macfie and Ingram (72), Mendelson (79); 1923 Joekes and Simpson (50), Pijper (91); 1926 Kotkis, Washowiak, and Fleisher (58); 1929 Craig (27), Marett (75); 1931 Warr (130); and in 1937 R. and J. Flinn (34). As a rule, the fungus was then looked upon as a secondary invader to Koch's bacillus, while one or another considered they had reason to assume the contrary, Pijper (91), R. and J. Flinn (34). In his paper in 1929 on yeast-like fungus forms in chronic lung infections Marett tells us, that in his systematic differential examinations of sputa from 304 patients, he had found 62 to be negative both to tubercle-bacillus and fungus infection; of the remaining 242 patients, 6.61 per cent were infected with tubercle bacilli only, 27.27 per cent

with mixed tubercle bacilli and fungus of yeast-type, and 65.11 per cent only with the latter. Marett emphasizes the importance of the fungi so strongly, that he says "that in cases in which fungi are not present the patient is usually not so much a sufferer as a carrier of tubercle". Kristenson (60) who in 1940 examined sputa from patients suffering from pulmonary tuberculosis (104 cases) and from patients without any clinical signs of actual tuberculosis (174 cases) found a yeast-like fungus in 2 per cent of the first group and in 5 per cent of the latter.

Several animal experimental investigations have been carried out for the purpose of ascertaining the pathogenity of yeast-like fungus strains isolated from sputum from cases of pulmonary affections. Most suitable as test-animals seem to have been rabbits, rats, and mice, while monkeys and guinea-pigs have sometimes proved susceptible, but dogs not at all. The results diverge to a high degree. Pijper (91), who in 1923 carried out tests with yeast-like fungus strains from cases of broncho-moniliasis, found that a quantity of 2,000 million fungus cells injected intravenously into a rabbit with great certainty killed the same within 24 hours, and after about one week, if half the dosis was used. Smaller doses occasionally caused death, but usually the animals remained alive for weeks, showing very marked emaciation and loss of power in their hind limbs. If such an animal died after some weeks the fungus could be found in sections of the organs. The most remarkable thing in such cases was *the absence of marked inflammatory changes* in such organs. It looked more as if the fungus had been quietly growing in the organs, than that a real parasite had gained access. — Similar effects, Pijper points out, have been recorded after subcutaneous injections of blastomyces by several authors. — In his inoculation tests into rabbits Pijper obtained a high agglutinative titre and very marked powers of complement fixation, but if in immunisation killed fungus was used, the result was negative, no matter how often and how large doses were used. Such results were also, according to Pijper, seen by Magalhaes. As a general rule such large doses seem to have been used, that the animals in the case of intravenous and intrapulmonary inoculation died within a short period.

After intravenous inoculation of yeast-like fungus strains isolated from broncho-moniliasis into rabbits mycotic metastases have generally been obtained in most of the organs in the form of white,

raised nodules the size of about a pin's head. Redaelli (97) in 1924 obtained always such in the heart muscle, the spleen, the suprarenals, and the abdominal lymphatic glands. According to Redaelli the fungus elements, being introduced into the circulation, either act as an embolus, and as such stay in the small vessels, obstructing them, or they form a small thrombus in the medium sized vessels, and from that fungus cells can develop and penetrate through the walls of the vessels into the surrounding tissues. The nodules consist in the centre of cells of chiefly haematogenic and lymphogenic origin, and in the peripheries of histogenic origin. Apart from the formation of nodules he also mentions what he calls distant reaction in the organs, degenerative changes, especially in the heart and liver. Besides fatty degeneration he observed zones of real necroses in the liver. The nodules may, especially in the lungs, after both intravenous and intrapulmonary inoculation of the fungus in question, be manifestly reminiscent of tubercles. They consist then of masses of small, white cells and polymorphonuclear leucocytes which decrease in numbers peripherally, and are surrounded by a ring of epithelioid cells, sometimes together with a few large, multinucleated giant cells, and also now and then foci may show caseous formation in the centre. Nodules resembling tubercles were noted even by other investigators, e. g. by Grossi and Balog (40) in 1929 (intrapulmonary inoculation into rats). Stokes, Kiser, and Smith (120), who in 1930 inter alia inoculated rats subcutaneously and intraperitoneally with fungus from broncho-moniliasis, obtained histological changes which fully agreed with such as occur after inoculation of tubercle bacilli. The last-mentioned authors point out that the only difference between the changes obtained and tuberculous ones was, that they were able in the foci to prove fungus elements, but no tubercle bacilli. Magrou (74) relates in 1919, that he had inoculated rabbits intravenously with a yeast-like fungus from a case of pulmonary affection, and in one rabbit, which died after 5 days, found in the kidneys inflammatory nodules of polynuclear cells, and in the centre a felting of mycelial filaments surrounded by a ring of acid-fast, claviform formations.

Lim, Kurotchkin, and Wu (70) inoculated in 1930 some rabbits intratracheally with a yeast-like fungus strain from broncho-moniliasis, but without result. It was not until after intravenous inocula-

tion of chaulmoogra-oil[1], which destroyed the parenchyma in the lungs, that the fungus cells were able to grow. In various inoculation tests into monkeys with a yeast-like fungus strain from a fatally terminating case of broncho-moniliasis, Reimann and Kurotchkin (104) in 1931 obtained a negative result, although for the destruction of the lung-parenchyma pulverised glass, chaulmoogra-oil, etc. had been used.

Many other animal experimental investigations have been carried out with yeast-fungus forms from cases of pulmonary affections, which nevertheless would take far too much space to record, for which reason the quotations must serve more as illustrating examples of the results of investigations in question, than an exhaustive description.

Although, as is seen from the preceding statements, the fungus in question has many times been met with in tuberculous material containing Koch's bacilli, or in changes which are strongly reminiscent of such as occur in tuberculosis, and also that inoculation into animals many times has caused changes that agree with tuberculous ones, *the fungus has not been brought into any connection whatsoever with tubercle bacilli*, although this is extremely near.

The first to emphasize a real relation between a fungus of yeast-oidium type and Koch's bacillus, was Reenstierna who, through keen observations proved that the fungus in question was able to produce acid-fast rods (98—103). His way to this conception passed via observations of the causative agent of leprosy.

Thus, in February, 1912, he isolated a mycotic non-acid-fast micro-organism from the blood of a patient suffering from nodular leprosy in the acute stage. Growing in a special fluid medium, it formed, besides solitary large prickly cells, chiefly long chains whose links partly resembled small yeasts (Pl. III, fig. 1) and, furthermore, were able to acquire acid-fastness, thus resembling Hansen's bacilli (Pl. III, fig. 2). His cultural and animal studies convinced him that the micro-organism in question was the fungus that causes leprosy, and of which the bacillus of Hansen is merely a part.

The bacillus of Hansen and that of Koch being as like each other

[1] This substance causes namely, as Frazier and Chen (38) have shown in 1930, extensive lung damage when injected intravenously into rabbits.

as twins, Reenstierna concluded that a "mother-fungus" of the latter must also exist, and that the same should also be yeast-like.

He then began, in June 1912, to examine different tuberculous material and was able to establish the occurrence, in sputa and uretero-catheterized urines, of a fungus of yeast-oidium type which was isolated and studied in *single cell* cultures. In October of the same year he made extremely interesting discoveries: In two single cell cultures (glycerin boullion) of the fungus — originating from the sputum of a patient suffering from pulmonary tuberculosis — there had *appeared* in between the non-acid-fast fungus cells, acid-fast rods which morphologically and tinctorially were indistinguishable from the bacillus of Koch. Moreover, as the plates in one of Reenstierna's papers show (103) — also reproduced in a paper by Gullberg (41) — the rods were disposed in small groups, the size and shape of which were, in parts, those of an ovoid fungus cell. Finally, fungus cells in a state of disintegration were seen, leaving behind as a remnant different bacterial elements, i. e. the finest, almost invisible powder[1], cocciform organisms, diphtheroids, as well as acid-fast rods of tubercle bacillus type. Briefly, the origin of the acid-fast rods had been proved to be the fungus. Inoculations into guinea-pigs with cultures of the fungus did not result in any tuberculous changes of the inguinal glands and internal organs. Some of the animals, however, died *cachectic*. Reenstierna himself thinks that the acid-fast rods in question are to be considered as merely pre-stages of Koch's bacillus.

Reenstierna's observations were confirmed and extended by Gullberg (41) in the years 1933—1935. Thus, in 1933, he isolated a non-acid-fast fungus of yeast-oidium type from the sputa of two patients suffering from pulmonary tuberculosis, and also from the blood of a patient with high fever and erythema nodosum in his history. These three strains were studied in *single cell* cultures.

In glycerin boullion culture of two of the strains (one of them originating from the sputum and the other from the blood) there appeared, more than a year after their isolation, acid-fast rods of Koch's type. In the smears Gullberg found inter alia fungus cells, partly in granular disintegration, and in or around the destroyed

[1] Reenstierna draws special attention to this powder, according to him no doubt filterable, passing "les bougies et le placenta (hérédité de la tuberculose; . . .) . . ."

cells there were fine, pulverised or amorphous masses which in some pictures seemed to differentiate into a net-work of rods, some of which were acid-fast, and in appearance could not be distinguished from Koch's bacilli (Pl. IV, figs. 5 and 6). The acid-fast rods occurred also in small agglomerations of approximately the same size and outline as an ovoid fungus cell, but also in a remarkably large quantity, formed into balls.

Before acid-fast rods were encountered in the cultures, 10 rabbits were inoculated intravenously, eight of them dying between one week and 7 months later. Six of them showed only non-specific changes of the organs, while in the lungs of the seventh were seen pneumonic foci and septic infarcts. In the areas not occupied by infarcts were seen in some places proliferations of epithelioid cells grouped in such a way that pictures occurred which to a certain extent were suggestive of those found in tbc. The remaining rabbit, which died $3\,^1/_2$ months after the last inoculation, showed at the autopsy *typically general tuberculosis of internal organs*, with acid-fast rods which, on being isolated from the spleen, in glycerin bouillon showed the same surface growth as Koch's bacilli. Guinea-pigs inoculated with this culture always died of typical tuberculosis. On the other hand, only one of the guinea-pigs inoculated subcutaneously with a suspension in NaCl-solution of the crushed spleen from the rabbit in question, died of tuberculosis.

From the afore-said cultures in which acid-fast rods had appeared, were inoculated altogether 8 guinea-pigs and 2 rabbits. One guinea-pig, which was inoculated subcutaneously, showed 20 days later at the spot of inoculation a subcutaneous abscess the size of a walnut, which on being punctured proved to contain yellowish-green pus with acid-fast rods of Koch's type. The animal died *cachectic* two months after the inoculation, but manifested, apart from acid-fast rods in the lungs, no organic changes which indicated tbc. 4 fresh rabbits were inoculated with the punctate. Two were still alive after twelve months, while two died after respectively 6 and 11 months without any signs of tbc. but one of them with *cachexy*. 3 guinea-pigs were inoculated subcutaneously with pieces of crushed organs emulsioned in an NaCl-solution, these pieces originating from the animal with an inguinal abscess, but no tbc. was manifested. One of them died *cachectic*.

From a 14 months old boy suffering from pneumonia and empyema,

and who shortly after birth had been B. C. G.-vaccinated, Gullberg found in smears from the empyemic pus yeast-like fungus cells and acid-fast rods. The latter, but not the former, were obtained in culture where they grew as Koch's bacilli and, inoculated into guinea-pigs, caused tuberculosis.

Plá y Armengol, 1931 (94) saw yeast-like elements in a 53 days old tubercle bacillus culture. Similar ones seem also to have been observed by Minchin according to Plá y Armengol, who states in his paper "... die von Reenstierna, von uns und von Minchin beobachteten hefeförmigen Formen".

Hollström, 1942, (48) in a 20 days old glycerin bouillon culture of a peculiar tubercle bacillus strain (capable of producing non-acid-fast, diphtheroid rods) was able to establish the appearance of yeast-like fungus cells, some of which contained acid-fast elements.

It is of great interest that in 1941 Schaumann (109), and also Schaumann and Hallberg (110), have been able to establish in organs from cases of Lymphogranulomatosis benigna (Schaumann's disease) the occurrence of fungus cells, which they assert as being quite similar to those described by Reenstierna in 1912, and, furthermore, that the authors in the organs in question, apart from such fungus cells, have also found acid-fast rods with the same morphological and tinctorial properties as those of Koch's bacillus. These findings are especially interesting in view of the fact that L. B. is by Schaumann regarded as being a disease of a tuberculous nature, though it does not seem to cause positive tuberculin reaction. The findings of acid-fast rods and fungus cells made have been enabled, or at least largely facilitated, by Hallberg's new method of staining tubercle bacilli (42). For with this method of staining a good many fungus elements prove to be acid-fast and the tubercle bacilli look larger and more distinct and are easier to find.

In this connexion a disease in cattle, which in American literature passes under the name of "skin lesions", is of interest. It is characterized by cutaneous and subcutaneous resistances, generally localised to the extremeties, the neck and the belly, which display microscopically a picture that has great similarity to, and in many cases agrees with, genuine skin tuberculosis. They contain almost constantly acid-fast rods, which often to a high degree have the appearance of tubercle bacilli. They have been found very difficult to cultivate, and on inoculating into animals have not proved to be pathogenic,

nor have they called forth positive tuberculin reaction, which is mostly found in animals with skin lesions.

But it is of special interest that Hedström (44), as the first, in 1941, succeeded in isolating a yeast-like fungus from these skin lesions and corresponding lymph glands, which in appearance, according to the description given, seems to agree with that described by Reenstierna. Hedström has been able to cause with this fungus on inoculation into cattle changes of tissue of the same kind as in "skin lesions", and to a large extent also tuberculin allergy.

Even another fungus than the one of yeast-oidium type has been brought into direct connexion with Koch's bacillus. Dostal (30) in 1913 has namely by experimental research considered himself able to prove the origin of an aspergillus from tubercle bacilli via non-acid-fast, coccus-like micro-organisms. Single cell culture of the aspergillus in question caused, on inoculation into *one* guinea-pig, tuberculous changes containing typical Koch's bacilli. An NaCl-emulsion of crushed organs from this animal caused, on inoculation into two guinea-pigs, in its turn tuberculosis. Unfortunately, no pictorial material of Dostal's meticulously described tests is available.

According to Weissfeiler (131), Enderlein, too, is reputed to have obtained a transition of tubercle bacilli in aspergillus and also to have advanced a theory in which he vindicates the opinion that the bacillus of Koch originates from an aspergillus. Weissfeiler does, however, not render an account of those findings upon which Enderlein is basing his conception.

Already in 1883, one year after the discovery of the tubercle bacillus, Babes and Cornil (4) as well as in 1884 Petrone (89) observed atypical tubercle bacilli, ramified forms, as well as such with filament and spadix formations, which induced the last-mentioned to assume a connexion between Koch's bacillus and groups of organisms that are on a higher level than the bacteria. Since that time a great number of author's have emphasized the pleomorphism of the tubercle bacillus which is evident from the monographs by Kahn (51), Roman (105), Hedvall (46), Weissfeiler (131), and others. Nevertheless some few investigators adhere to the monomorphism of Koch's bacillus, e.g. Lange (65).

Several investigators have assumed a kinship between the

tubercle bacillus and the actinomycetes, or organisms closely akin to them. — An expression for the kinship between Koch's bacillus and fungoid organisms is the term Mycobacterium tuberculosis introduced by Lehmann and Neumann (132) in 1896. — Dalous (28) in a work of the year 1901 includes the tubercle bacillus amongst these fungi, and Lieske (69), in his "Morphologie und Biologie der Strahlenpilze", 1921, emphasizes that Koch's bacillus is so closely related to the actinomycetes that it is impossible to draw a distinct line of demarcation between these two groups of organisms. In 1930 Karwacki (52) and Kedrowsky (53); in 1931 Fejgin (32) and Møllgaard (83); and in 1935 Weissfeiler (131) observed the development of typical actinomycetes from tubercle bacilli; and in 1930 Karwacki (52); in 1931 Møllgaard (84), Triuss and Politowa (127); and in 1936 Kedrowsky (54) obtained tuberculous changes in inoculation into animals. Weissfeiler (131) draws from these tests the conclusion that the tubercle bacillus is a typical actinomycete, which in its parasitic form occurs mostly as an acid-fast rod.

The actinomycetes do not manifest kinskip only with Mycobacterium tuberculosis but also with the leprosy bacillus and other acid-fast bacteria, some of which occur as saprophytes in nature. They have by Lehmann and Neumann (67) been placed together into the group Mycobacterium. It has often been found very difficult to distinguish the so-called acid-fast saprophytes from Koch's bacillus. Some of them grow on artificial media more quickly than Koch's bacillus, and, as Schlossberger and Pfannenstiel (111) have shown in 1920, both in living-room temperature and at a temperature of 50°—55° C, in which respect they agree with Mycobacterium tuberculosis, typus Galinaceus. Others have been found uncultivable. The acid-fastness of some species has been described as less pronounced, but one can also in the tubercle bacillus find great variability in this respect. Nor can the two groups of Bacteria be safely separated in regard to form. In inoculation into animals of the acid-fast bacteria occurring in nature, their pathogenity has been very varying. Some of them have been able to cause changes in the test animals, which fully agree with those obtained in inoculation of tubercle bacilli, which has been proved inter alia by Moeller (82) in 1899. On the other hand, tubercle bacillus strains have been observed with considerable variations in their pathogenity, e. g. as Pinner (93) has shown in 1932. It is highly interesting that in 1921 Kolle, Schloss-

berger, and Pfannenstiel (57) were able, for eight separate saprophytic, acid-fast strains to show by repeated guinea-pig passages increasing virulence, which agree fully with those caused by genuine tubercle bacilli. Similar observations have, according to Lehmann and Neumann (67) in 1925, also been made by Sanfelice (106) and Schröder (112). There is no certain serological reaction to distinguish the different acid-fast micro-organisms.

The opinion has been advanced that the tubercle bacilli are the parasitic form of acid-fast saprophytes growing "wild" in nature. Perhaps a way leads from human-bovine via avian and cold-blooded types of tubercle bacilli down towards the acid-fast saprophytes in nature? But this must, according to Mellon (78), still be regarded as a theory, for which evidence is lacking.

The location of the tubercle bacilli in the fungoid-bacterial system is still unclear. Castellani (20) has classified them amongst the Fungi Imperfecti, but other systematicians amongst the bacteria. Although mycobacterium tuberculosis generally occurs in Bacterial form, it may, occasionally, show mycotic qualities, which, as stated in the preceding, has induced several investigators to assume kinship between the tubercle bacillus and higher micro-organisms. This old conception is to be found in a modernized form in Pribram's (96) statement in 1927, that "Bacterium tuberculosis itself is a transitional form, not between different bacteria, but between the whole genus Bacterium and the genus Hyphomyces (fungi imperfecti)".

OWN INVESTIGATIONS.

CHAPTER II.

The Cases from which the Fungus Strains have been isolated.

Out of the five fungus strains with which I have carried out my research, three have been isolated from gastric lavage from patients who, for the purpose of ascertaining possible pulmonary tuberculosis, had been sent to the Central Sanatorium at Sandträsk (Physician in Chief: Dr. B. Carstensen). The two remaining strains have been obtained from the sputum of patients with clinical diagnosis of pulmonary tuberculosis, who were being treated at the Central Sanatorium at Upsala (then Physician in Chief: Dr. A. Kristenson). The histories of their diseases are briefly related here.

Case I. S. P., a woman 40 years old. In the year 1937 insidious trouble in the form of aching in the finger-, hand-, and knee-joints, as well as sometimes sensitiveness to pressure over the finger-joints. In the winter of 1938—39 she was troubled with cough with yellowish or white expectorations, but never mingled with blood, especially in the mornings. On examination at the Central Sanatorium at Sandträsk, in March, 1939, the following diagnosis was made: Positive tuberculin reaction. B.S.R. 48 mm/1 hour. X-ray film of the lungs shows within the right Scl–I_1 and in the left Scl irregular spottiness and stripiness, probably fibrous remnants from an earlier process, and at the forward edge of the right C_I a small calcination. Thus the X-ray examination did not supply any certain criterion for an *active* tuberculous process. The patient was taken into the same sanatorium in July, 1939. From the income status is ascertained: Pale, lean woman, grown old before her time, with habitus astenicus and typical sideropenial appearance. Heberden nodes. Indeterminate "rheumatic" ache in the joints, as well as here and there in other parts of the body. Gastro-intestinal disturbances. B.S.R. 36 mm/1 hour. No fever. Stethoscopically some unclear vesicular respiration can be distinguished over the apices of the lungs, but otherwise nothing remarkable. No sensitiveness to pressure over the finger-joints. No roentgenological change of the lung-picture since the last examination. During the fifty days' stay at the hospital the sputum has been examined seven times, no acid-fast bacilli being found on any one occasion. A cultivation on Hohn-

medium and inoculation into guinea-pigs were made with gastric lavage treated with sulphuric acid. A *fungus of yeast-oidium type* was obtained in pure culture. No acid-fast rods. The test in guinea-pigs was negative. B.S.R. kept all the time round about 20—25 mm/1 hour, and the state of the lungs was unchanged.

Clinical diagnosis: *Tbc. pulm. (peracta?) + Sideropenia + Osteoporosis.*

Case II. K. P., 25 years old woman. Treated in 1928 at the Barkåkra Coast Sanatorium for lymphadenitis colli tbc. and kerato-conjunctivitis phlyctaenulosa oculi dexter. When, in 1934, the glands on the neck had begun to increase in size, she was in July of the same year received at the Central Sanatorium at Sandträsk, where she stayed 2 $^1/_2$ months, and was subsequently examined repeatedly. The examination in 1934 gave the following result: Slight cough but no expectoration. B.S.R. 12—8 mm/1 hour. X-ray film of lungs shows a right-side pleural rind, which extends upwards over the apices of the lungs. In the left Scl I_1 a number of small, lime-dense spots. Above the left hilus pronounced bronchioectatic signs. Congestion of the pleura possibly also in the left Scl. Minor calcareous foci in and below left hilus. On an examination in 1937 the following findings were made: Glands the size of a walnut on the neck. Has lost 10.4 kilos in weight. Lung picture, however, still unchanged since previous examination. In July, 1938, X-ray films of the lungs show a triangular congestion in the lower edge of the bronchioectases, but otherwise the picture is unchanged. B.S.R. 20 mm/1 hour. The patient became acutely ill in May, 1939, with a stitch in the right side of the chest and fever up to 41° C, and shiverings. No cough. After 2 days the temperature dropped to 38° C, and after a further couple of days it was normal. In July of the same year she was received at the sanatorium for observation, the picture of the lungs then being apparently unchanged. A cultivation on Hohn-medium from gastric lavage treated with sulphuric acid resulted in the growth of a *fungus of yeast-oidium type* in pure culture, but no acid-fast rods, and inoculation into guinea-pigs gave a negative result. B.S.R. 10 mm/1 hour. In 1940 the triangular congestion above the left hilus seems to have increased, but the picture otherwise to be unchanged. In May, 1941, there were palpated on the left side of the neck two glands, one the size of a walnut and one the size of a bean, not perfectly insensitive. Two months earlier, they had been considerably larger and tender. X-ray films of the lungs then show that the congested portion had disappeared and the picture in all particulars is identical with that of 1934. Apart from a casual congestion (atelectasis?) the picture of the lungs has thus been unchanged and not given any certain criteria for *active* tuberculosis.

Clinical diagnosis: *Tbc. pulm. (peracta?) + Bronchioectasiae lob. sup. dexter.*

Case III. G. E., a woman 23 years old. During the years 1933—1935 and 1939—1942 she had several times been treated at the medical clinic of the Academic Hospital in Upsala and at the Central Sanatorium there under the diagnosis of *tuberculosis.* The state of her lungs had been controlled in the in-

tervals. She has had several small haemoptyses but never any tubercle bacilli in her expectorations, and rarely fever. B.S.R. has as a rule kept between 10—20 mm/1 hour. Tuberculin positive. X-ray films of the lungs in 1933 show enlarged and congested hilus shadows and minor calcinations in the parenchyma. In the right Scl-I_1 cloudy confluent spots, which gradually congested and grew better delimited. The picture then mainly unchanged until 1937, when a floccula appears in the left Scl and a dissemination of congestions takes place within the right I_1–I_4. The state of her lungs is then on the whole unchanged until the month of May, 1939, when in an X-ray film of the lungs one can see distinct progress, in that laterally to the right C_{II} there is a cavity(?) the size of a plum, which afterwards in a film in August of the same year shows a distinct diminution, and in films in September and November of the same year cannot be noted with certainty. The condition of the lungs otherwise unchanged. In October, 1940, the changes show strong retrogression. A streak marks the lower limit of the right upper lobe, which is drawn up to C_{II} and in a film in February to C_I. Pneumothorax started on the right side in February, 1942, and has since then been maintained. The lung collapsed rather well downwardly, but was upwardly distended by broad adherences. After burning off these the whole lung shows very good collaps. No cavity can be observed there. The condition on the left side is unchanged. In September, 1939, there was found in the sputum a *fungus of yeast-oidium type*, which was pure-cultivated on ordinary agar. In 1942 it is still present in the sputum fairly plentifully, but no acid-fast rods have ever been observed there. On inoculating into guinea-pig in 1939 with sputum a negative result was obtained.

Clinical diagnosis: *Tbc. pulm.*

Case IV. A. L., a man 41 years of age. By reason of pulmonary tuberculosis in his family an examination of his lungs was made at the Central Dispensary at Luleå in June, 1939. In the film are then seen within both Scl in parts of more recent date, in parts softer, in parts confluent, apparently calcareous spots (the film, however, not perfectly marked throughout). In order to ascertain whether he was a carrier of tubercle bacilli, the patient was in June of the same year taken into the Central Sanatorium at Sandträsk. In an X-ray film of the lungs there appear then materially only remnants of an earlier process. Nothing in the picture speaks for activity. B.S.R. 5 mm/1 hour. In cultivation on Hohn-medium from gastric lavage treated with sulphuric acid a *fungus of yeast-oidium type* was obtained in pure culture. No acid-fast rods. Inoculation into guinea-pig gave a negative result. In controlling the lungs in January, 1940, no changes of the lung-picture visible.

Clinical diagnosis: *Tbc. pulm. peracta?*

Case V. V. S., a 39 years old woman. Under treatment for tuberculosis of both lungs at the Central Sanatorium in Upsala from 2nd January, 1939, until 8th November, 1939, when she died. During the whole time the patient had tubercle bacilli in the sputum as well as a *fungus of yeast-oidium type*, which was pure-cultivated on ordinary agar.

Anatomo-pathological diagnosis: *Tbc. pulm. amb. cum caverna multiplex pulm. dexter + Laryngitis et Tracheobronchitis tbc. + Tuberculosis intestini + Steatosis hepatis + Amyloidosis lienis + Degeneratio parenchymatosum organorum.*

In sections from the tuberculous lung foci plenty of tubercle bacilli are seen and rather sparse, yeast-like fungus elements, solitary and in minor groups.

Of the five cases described three (I, II, and IV) were observed for suspected pulmonary tuberculosis, showing roentgenological changes of the lungs but no proved active process. Case III has always been looked upon as one with lung tuberculosis, though no tubercle bacilli have at any time been found in the patient's sputum. Case V has from the beginning been a serious case of progressing lung tuberculosis with tubercle bacilli in the sputum. *The fungus isolated from the five patients was of the same type as Reenstierna's of 1912.*

CHAPTER III.

Isolation of the Fungus Strains and Reproduction of Single Cell Culture.

The fungus strains have been denoted by the same numbers as the cases from which they originate. Nos. I, II, and IV (from gastric lavage) were isolated on Hohn-medium, Nos. III and V on ordinary agar. Thereafter 10 spreading passages on agar plates were carried out, each time with re-inoculation from a single colony. Then the five strains were transferred into slanted agar tubes and re-inoculated on the average once every month. Having reached their 10th generation, about one year after their isolation, the fungus strains were used for the preparation of *single cell* cultures.

For the purpose of obtaining a single cell culture a Chambers' micromanipulator with inverted microscope was used. From the research material, fresh fungus cultures on agar, were emulsioned smaller quantities, less than $^1/_2$ normal loop in 100 cc of a physiological NaCl-solution, after which drops of the same were transferred to the slide of the micromanipulator and examined. When a drop containing only *one* fungus cell was met with, this was transferred to an agar plate, where a *single* colony then developed. The techni-

cal process is analogous with that employed by for example Kahn (51). The latter worked, however, with a hanging-drop, while I, on account of the microscope used, had the advantage of a lying drop. On transferring to or respectively removing, from the slide, capillary tubes were used, which had been protracted automatically by means of a Du Bois needle-pulling machine, and then bent over a micro-flame in a suitable manner. The apparatus used is described in detail in Mc Clung's Microscopical Technique (77).

All parts of the apparatus which might come into contact with the fungus emulsion were sterilised immediately prior to the commencement of the tests by boiling for 1 hour. The slides were dipped also in Spir. conc. and fired. No other growth than the fungus in question has ever been obtained.

The *single cell cultures* thus obtained were then cultivated for 10 generations on ordinary slanted agar, before cultivation on media suitable for tubercle bacilli or animal tests were carried out.

CHAPTER IV.

Cultural, Tinctorial, and Biochemical Properties of the Fungus.

At a temperature of 37° C the fungus grows on the surface of ordinary agar or glycerin agar within a period of 24 hours to round, slightly arched, white colonies the size of a pin's head, with moist surface (S-form). The microscopic examination shows that some of the colonies consist only of round and ovoid cells (as a rule 4—6 μ in diameter), and furthermore others of elongated ones, which grow out from the border, thus forming mycelial branches (Pl. I and II). After only a few days' growth the size of the fungus cells may vary in a high degree. The mycelial colonies occur more often inside the agar (Pl. II). At living-room temperature fairly good growth is obtained, though it proceeds more slowly than at 37° C. On solid substrata containing sugar, especially maltose agar, the fungus grows rapidly and luxuriantly, forming a cream-coloured mat (Pl. XXII, fig. 2), which, when the culture has become 2 to 3 weeks old, may show a puckered or relief-like surface (R-form). A dissociation in R-form may, however, also now and then occur immediately (Pl.

XXII, fig. 3). On Hohn- and Löwenstein-media the fungus grows much more slowly than on the other substrata mentioned, and may then present a picture which displays great similarity to that occurring in bovine tubercle bacilli culture.

In glycerin bouillon, too, growth is obtained in 24 hours. Generally, the fungus grows in the deeper layers of the medium as cloudy, stringy masses. The masses of fungus can, however, grow along the sides of the flask and out on the surface of the glycerin bouillon, forming a pellicle of finely meshed network, from which long cell conglomerations may suspend like rosaries or garlands. On merely lightly shaking the flask, the superficial layer of fungus drops down to the bottom. The fungus may sometimes show a granular growth on the bottom of the flask, making the medium turbid. It consists then merely of round and ovoid cells. The degree of acidity of the medium seems to play a small part for the growth of the fungus, but a poorer growth is obtained on a strongly basic medium than on a strongly acid one, and a moderate degree of acidity seems to be more favourable. In varying the glycerin content from 3 to 15 per cent no difference in the manner of growth, or the rapidity of growth, could be noticed. Nor did any difference become manifest when cultivating under aerobian or anaerobian conditions. In none of the different fungus cultures investigated could sexual reproductions be demonstrated.

In 24 hours' cultures the fungus cells are generally acid-fast on staining according to Hallberg (42) as well as gram-positive, but some of the cells lose within the next few days completely, or partially, their acid-fastness, so that for example in a 7 days' culture one may find all transitions between purely acid-fast and non-acid-fast ones. The gram-positiveness, too, decreases gradually, as does also, though more slowly, the general stainableness. On staining according to Ziehl-Neelsen, without counter-staining, the fungus is as a rule in fresh cultures faintly rose-coloured, with a frequently excentrically recumbent, unevenely outlined, acid-fast "nucleus". Methylene blue counterstain mostly conceals the red or rose-coloured stain (Pl. III, fig. 3), so that in only a minor number of the fungus cells the red "nucleus" is visible, and is then sometimes able to manifest itself as a faintly reddish cap (Pl. V, figs. 4—6). When staining in general, and especially older cultures, remarkably great variations of colour may be obtained. When cultivating on a blood agar

plate no haemolysis results, and in ordinary gelatine no colliquation.

Fully two years after the isolation of the fungus strains *preliminary* tests of their behaviour towards different species of sugar were carried out, as is shown by the subjoined table. (No test of acid formation was made.)

Strain	I	II	III	IV	V
Glucose	+	+	+	+	+
Galactose	—	—	—	—	—
Maltose	+	+	+	+	+
Saccharose	—	—	—	—	—
Laevulose	+	+	+	+	+

\+ = development of gas Sugar content 0.5 %
— = no development of gas

The five strains disintegrated glucose, maltose, and laevulose, in which according to Castellani and Chalmers (23) they agree with *Monilia pinoyi*. In repeated tests about 6 months after the preliminary ones the below-mentioned carbohydrates were used in a solution of 0.5 per cent and with an admixture of Andrade's indicator (neutralized acid-fuchsin) in a concentration of 1 per cent and in a fermentation tube. — The medium in the tubes amounted to about 10 cc, and to each tube was added a normal loop of fungus from a fresh agar culture.

A. Monosaccharides
  a. Pentoses
    Arabinose
    Xylose
    Rhamnose
  b. Hexoses
    Glucose
    Laevulose
    Mannose
    Galactose
B. Disaccharides
  Saccharose
  Maltose
  Lactose
C. Trisaccharides
  Raffinose
D. Polysaccharides
  Starch
  Inulin
  Dextrin
E. Alcohols
  Glycerin
  Adonite
  Mannite
  Sorbite
F. Glucosides
  Salicin
  Aesculin

In no tube was obtained gas or acid formation, although good growth had developed in all tubes, and the definite reading was not made until after 15 days.

The fungus seems thus in the six months that had passed between the two tests to have lost its capacity to disintegrate species of sugar. Furthermore, we do not know if the results achieved in the preliminary tests are those that would have been obtained with the cultures, if such tests had been carried out with the fungus strains immediately after their isolation.

Appearance of Acid-fast Rods amongst the Fungus Cells.

In a glycerin bouillon culture from strain I it was possible after 35 days' growth at 37° C to discern only some solitary acid-fast, tubercle bacillus-like rods, while in another, which had been growing at 37° C for 30 days and then in living-room temperature for 32 days, a large accumulation of acid-fast, granular rods (Pl. IV, fig. 2) could be observed. In a glycerin bouillon culture of strain II were seen after 36 days at 37° C amongst the round and ovoid fungus cells faintly stained ones of which a couple contained granules, which were partly lying in diplo- or strepto-units (Pl. III, fig. 4). Furthermore, there occurred in smears taken after both 36 and 45 days rather plenty of non-acid-fast rods with acid-fast granules at the end (Pl. III, fig. 5), and accumulations where some of the rods were entirely acid-fast (Pl. III, fig. 6), and also groups of solely acid-fast, granular rods (Pl. III, fig. 8). Some of the acid-fast elements were filiform (Pl. III, fig. 7), and in one smear it seemed possible to see how an acid-fast, filiform rod issued from a fungus cell (Pl. IV, fig. 1). The long, acid-fast micro-organisms might sometimes appear as a net-work, which on Gram-staining was positive (Pl. III, fig. 11). There were also met with accumulations that could be fairly dense and consist of acid-fast elements, which, at least in their peripheries, displayed a distinct rod-shape (Pl. III, figs. 9 and 10). In two glycerin bouillon cultures of strain III there appeared acid-fast rods. In one of them were found in smears from the culture after 9 and 13 days' growth at 37° C around the fungus cells amorphous, yellowish-brown masses containing differentiated rods, partly of the same yellowish-brown colour, partly acid-fast and arched (Pl. IV, figs. 3 and 4). Furthermore, granular and semicircular elements (fig. 3) occurred, forming the outlines of light-blue stained, more or less disintegrated, fungus cells. Finally, some very large, brown, cystiform formations (fig. 4) could be ob-

served (probably swollen fungus cells in a state of disintegration). In the second culture from strain III, where the glycerin content was 15 per cent as against the usual 3 per cent, were met with after 12 days at 37° C acid-fast rods of the same appearance as Koch's bacilli, partly diffuse and partly in bundles (Pl. V, figs. 1 and 2). *In altogether five glycerin bouillon cultures from three out of the five strains originating from single cells there had thus from the fungus cells been produced acid-fast rods.* In a couple of glycerin bouillon cultures started from organs of rabbits inoculated with the fungus in question there occurred acid-fast rods (Pl. V, figs. 4—12 and Pl. VI, fig. 1). An account of these findings will be rendered in Chapter VIII.

In spite of the absence of acid-fast rods I wish to deal in this connection with a finding in a glycerin bouillon culture from strain I. In a smear from this, taken after 35 days' growth at 37° C, peculiar corpuscles, much larger than the surrounding fungus cells, were observed. With Hallberg-stain they were of a yellowish colour and plentifully provided with red prickles (Pl. V, fig. 3). As to shape and appearance, they agreed with the pine-cone-shaped formations which Hallberg — terming them briefly "cones" — had demonstrated in cultures of the fungus here concerned, in sputa of patients suffering from pulmonary tuberculosis, as well as in different tissues from Schaumann's disease (43).

CHAPTER V.

Inoculation of Agar Culture into Rabbits.

On account of the slight knowledge of the pathogenity and possible capacity of the fungus in question to generate in the animal organism acid-fast rods, it has from the beginning been possible to organize the animal tests only roughly. These have been carried out with varying doses of injection, intervals between inoculations, age of cultures, etc. After that, supplementary tests have been carried out. This holds good not only in regard to tests with agar cultures but also in the case of inoculation with other material. Morbid symptoms and organic changes in the animals inoculated have been mentioned briefly in connection with the tests, but described in causal statistics (p. 79) and summarised in Chapter VII. Furthermore, it has

been ascertained that inoculation into rabbit of the fungus strains in single dose, even $^1/_2$ culture, as a rule has not called forth any symptoms of disease or anything else than trifling organic changes. With a sufficiently large dose of inoculation of fungus culture the rabbit reacted, however, by a change of body temperature.

Series 1.

Five animals, R 691—695[1], one for each fungus strain, were inoculated intravenously with $^3/_5$ fresh slanted agar culture, and as control two, R 697 and 698, with the same quantity of common yeast fungus culture on maltose agar (originating from ordinary baker's yeast). With regard to the former group of animals, it could be noted: The temperature prior to the test was between 37° and 37.6° C, but rose within 24 hours to between 38.5° and 40.1° C, the temperature of four of the animals being over 39° C. After 48 hours there existed still some rise in temperature in the case of four of them, while the fifth showed a normal temperature. On the third, or at least the fourth, day all the rabbits showed a hypothermy with a temperature varying from 34.8° to 36.2° C. On the fifth to the seventh day after inoculation the temperature rose to normal. The animals were killed a month after the inoculation. No macroscopic and only trifling microscopic changes of internal organs.

The two rabbits inoculated with common yeast fungus showed analogous variations of temperature, though to a considerably minor degree. Their highest temperature was 37.9° and 38.6° C, respectively, the lowest 36.8° and 36.2° C, respectively, and one month after the inoculation the internal organs presented no change from those of non-inoculated rabbits. No appreciable changes in weight could be established in any one of the inoculated animals.

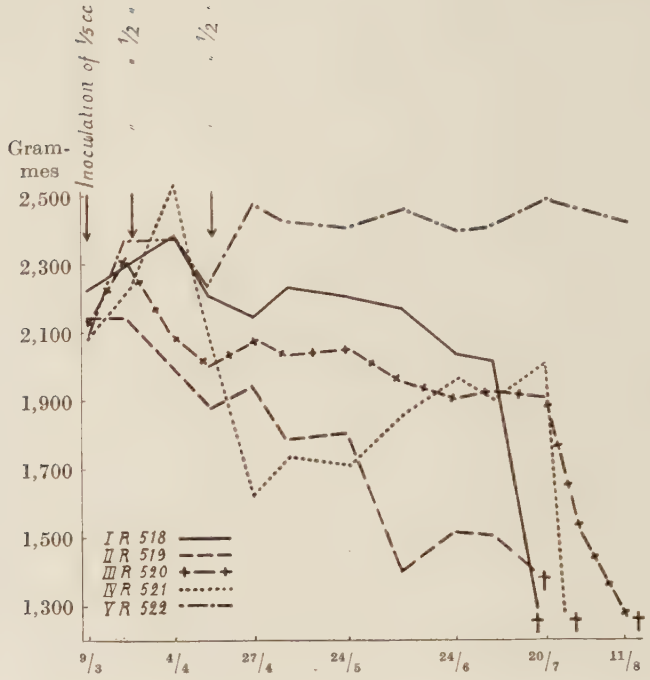
Series 2.

Intravenous inoculations, 3 times, of 2 days old agar cultures into 5 rabbits, one for each strain of fungus, at times and in doses that can be seen from diagram 1[2], where also the changes in weight of the animals can be followed.

[1] R stands for rabbit.

[2] The Roman figures state the fungus strain inoculated.

Diagram 1.



In diagram 1 it is seen that four of the animals died after[1] 3 to four months. They manifested pronounced *cachexy*, with losses of weight between 700 and 900 gr. The spleen, which was atrophic, was examined histologically in three of the animals, and displayed then in the pulp a moderate to strong disintegration of the blood. The organs otherwise showed only minor changes. Only in one case were remnants of fungus observed. The fifth animal, R 522, was killed after about $5\,^1/_2$ months, when it was found that no emaciation had taken place, nor any macroscopic changes in the internal organs.

Series 3.

Series 3 was started with 5 rabbits, R 552, 538, 539, 540, 541. They were meant to be inoculated intravenously 4 times with 2

[1] Unless otherwise stated the time always refers to the last inoculation.

to 12 days old cultures (one of the five strains for each rabbit). The chief purpose was to try to obtain acid-fast rods in the animal organism.

Two of the rabbits died within 15 days, one, R 538, of coccidiosis, and the other, R 539, of suspected such, both having been inoculated only once. R 540 was inoculated only twice on account of severe disturbance of balance, while the remaining two rabbits were inoculated 4 times with respectively $^1/_5$, $^2/_5$, $^2/_5$, and $^1/_2$ slanted agar culture. Since series 3 by reason of the intercurrent deaths had been thus decimated, a further 5 rabbits, R 553—557, were inoculated in the same manner.

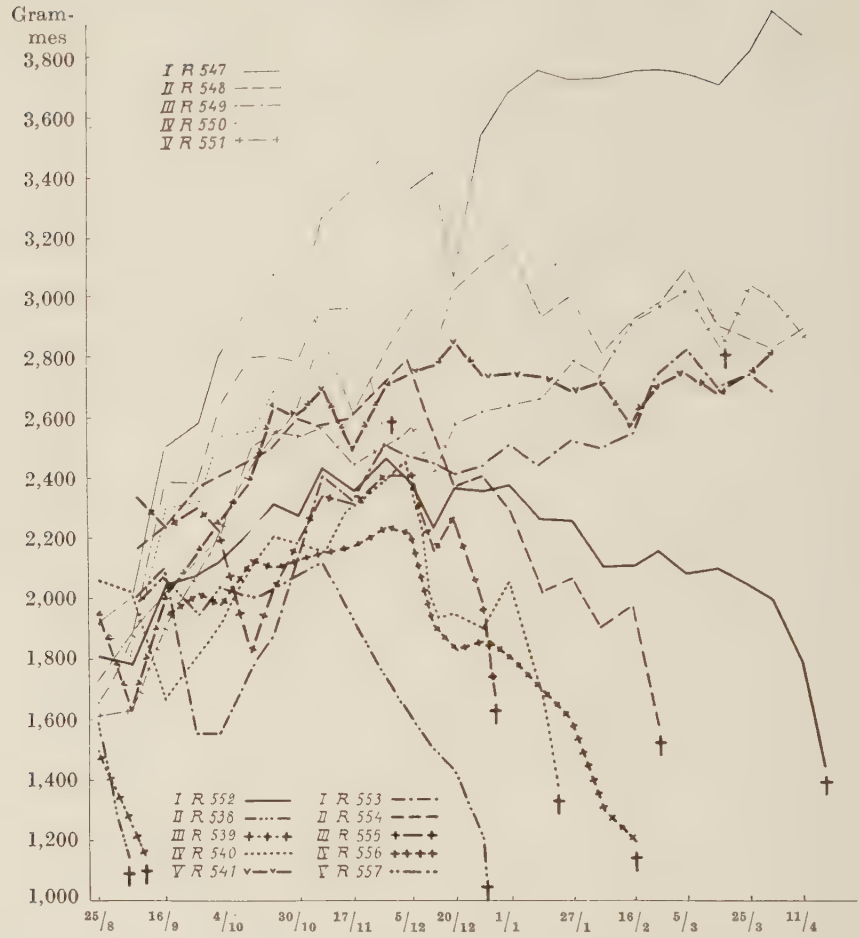
In order to ascertain at the same time whether the loss of weight manifested in four of the animals of series 2 was caused by the fungus cells as living organisms or by endotoxins, 5 rabbits, R 547—551, one for each strain of fungus, were inoculated intravenously 4 times with slanted agar cultures that had been killed by heat (58° C—$^1/_2$ hour). — Fungus cells thus killed do not seem to be changed in size or shape, and the endotoxins, according to what Buss (16) has observed in his tests with a yeast-like fungus, will not be destroyed at a temperature of 80° C for $^1/_2$ hour. All animals were inoculated in the course of one month.

Of the ten rabbits (see diagram 2) inoculated with live fungus culture, apart from the two previously mentioned ones, a further six died, these showing signs of disturbances in the central nervous system.

R 540, which after 9 days had shown serious disturbance of balance, after which there occurred gradually progressing paralysis of the hind part of the body, died *cachectic* within 5 months. At the autopsy the liver showed some single miliary, slightly fibrous foci, which in smears proved to contain some solitary fungus cells in a state of degeneration and "cones", but in sections only an increase in the periportal connective tissue. In the spleen was seen a faintly clear haemosiderosis. The brain displayed some ganglial changes.

In R 554 were observed after about $2\,^1/_2$ months moderate paresis of the hind legs and at the same time progressing bilateral infection of the eyes. It died after a further 2 months, during which it grew very lean (from 2,790 gr. to 1,580 gr.). The lungs showed diffuse bronchopneumonias with commencing necrotic disintegration.

Diagram 2.



In smears from here were found some solitary fungus cells fairly plentifully intermingled with "cones" and gram-negative rods. The abdominal organs showed only moderate inflammatory changes, but no findings of fungus cells or bacteria were made there. In the eyes were noted purulent changes, and in the brain were found, besides perivascular round cell infiltrations, a couple of necroses with concrement-like formations.

R 555, 556, and 557 died after respectively 3, 4, and hardly 3 months, the first-mentioned with reduced flesh on the body, and the two last ones *cachectic*. R 555 and 557 showed no changes of

internal organs, apart from a moderate haemosiderosis in the spleen of R 557. R 556 showed purulent bronchitis and bronchopneumonia, which proved to contain sparse fungus cells and plenty of bacilli of proteus type. In the central nervous system of the three rabbits were noticed diffuse, minor round cell infiltrations.

R 552, which after 9 weeks showed permanent disturbance of balance, and after a further 3 months progressing paresis in the hind legs, died about 2 months later. In one lung was noticed, just below the surface, a pneumonic focus the size of a hemp seed, which contained solitary fungus cells and minor fragments of cells. Besides considerable splenic atrophy, with strong haemosiderosis, the abdominal organs were otherwise without appreciable changes. In the brain were found, apart from perivascular round cell coatings, plenty of corpuscles of the same size and appearance as those described by Wright and Craighead (133) and Levaditi, Nicolau, and Schoen (68) in a form of stable encephalitis in rabbits, and by the three last-mentioned authors termed *Encephalitozoon cuniculi.*

In R 541 and R 553 disturbance of balance occurred first after eight months. They were killed $3\,^1/_2$ months later, when both were fairly well nourished. R 541 showed, besides diffuse perivascular round cell infiltrations in the brain, no appreciable organic changes, while R 553 showed moderate haemosiderosis in the spleen, a diffuse induration of connective tissue in the kidneys, and minor granulomata in the liver and brain.

Two of the rabbits inoculated with killed fungus culture died. One of them, R 549, died after 2 months of tracheitis with masses of mucus, which largely obturated the upper part of the tracheal lumen, but without macroscopic changes of internal organs, and the other, R 550, after hardly 6 months of pasteurellosis. Both were well nourished. R 551 presented after $2\,^1/_2$ months an infection on one side of the gluteal region, which healed in about one month. During this time the rabbit displayed no increase in weight. The remaining animals, R 547 and R 548, survived and had not lost any weight.

In the diagram there appears a considerable difference in the weight between the animals which died after inoculation with killed bacilli and those that died after inoculation with live cultures. The former weighed respectively 2,500 and 2,830 gr., the latter from 1,080 to 1,675 gr.

Simultaneously with the animals in series 3 were weighed 3 non-inoculated rabbits, and these kept their weight all the time within the limits of those inoculated with killed fungus.

CHAPTER VI.

Inoculation of Glycerin Bouillon Culture into Rabbits.

Intravenous inoculation of glycerin bouillon cultures in varying single doses have been carried out, without the results materially differing from those that had been obtained in analogous tests with agar cultures.

Series 1.

Five rabbits, R 526—530, one for each fungus strain, were inoculated twice intravenously, with 7 days intervals. The cultures had previously been centrifugated, after which the glycerin bouillon was pipetted off and replaced by the same quantity of a physiological NaCl-solution.

Two of the five rabbits, R 526 and 528, were killed after nearly six months. One of them had manifested a left-side cataract and moderate obliquity of the head. Both showed reduced flesh, but at the autopsy no macroscopic changes in the internal organs. Two rabbits died intercurrently of respectively rhinitis and coccidiosis.

R 527, whose initial weight was 2,100 gr, diminished after the second inoculation greatly in weight, so that one month later it weighed only 1,250 gr. Its general condition of health was so strongly impaired that it was expected to die. After another 14 days there were noticed on the belly two subcutaneous resistances. The rabbit gradually increased in weight, so that, when it was killed after about 6 months, it weighed 1,970 gr.

At the autopsy *macroscopically tuberculosis-like changes* were established *in the lungs, liver, and spleen.* The microscopical examination of sections from the lungs showed pictures which *in no small measure were reminiscent of tuberculosis.* There was found a peculiar granulomatous process with partly caseous, necrotising granulomata the size of nearly a pea, where the granuloma cells are epithelioid and frequently mixed with giant cells in large quan-

tities. In smears, the same as in sections of the lungs, there occurred *plenty of acid-fast rods of Koch's type* (Pl. VIII, fig. 1), as well as *sparse, degenerated fungus cells* and small gram-negative, bipolar rods. The liver and spleen presented diffuse, small granulomata with giant cells but without necrosis, yet with *acid-fast rods* (Pl. VIII, figs. 2 and 3), *which on being cultivated grew in pure culture.* (For cultures of the acid-fast rods and animal tests with these, an account is rendered in Chapter IX.)

Of the afore said two subcutaneous resistances on the belly, one still remained. On examination it was found to consist of a detritical mass containing *no acid-fast bacilli* but *small gram-negative, bipolar rods*, which on cultivating in blood agar plates grew as non-haemolysing colonies resembling dew-drops.

Such subcutaneous abscesses[1] containing similar non-acid-fast rods, but no acid-fast elements, occurred in 7 further rabbits, of which two had been inoculated with fungus culture 4 months earlier, and 5 were not inoculated. Furthermore, the animals that were killed two months after the appearance of abscesses, did not show any loss of flesh nor any macroscopic changes of internal organs.

There are thus no criteria for the acid-fast rods detected in the lungs, liver and spleen of R 527 being derived from the said stable infection.

Series 2.

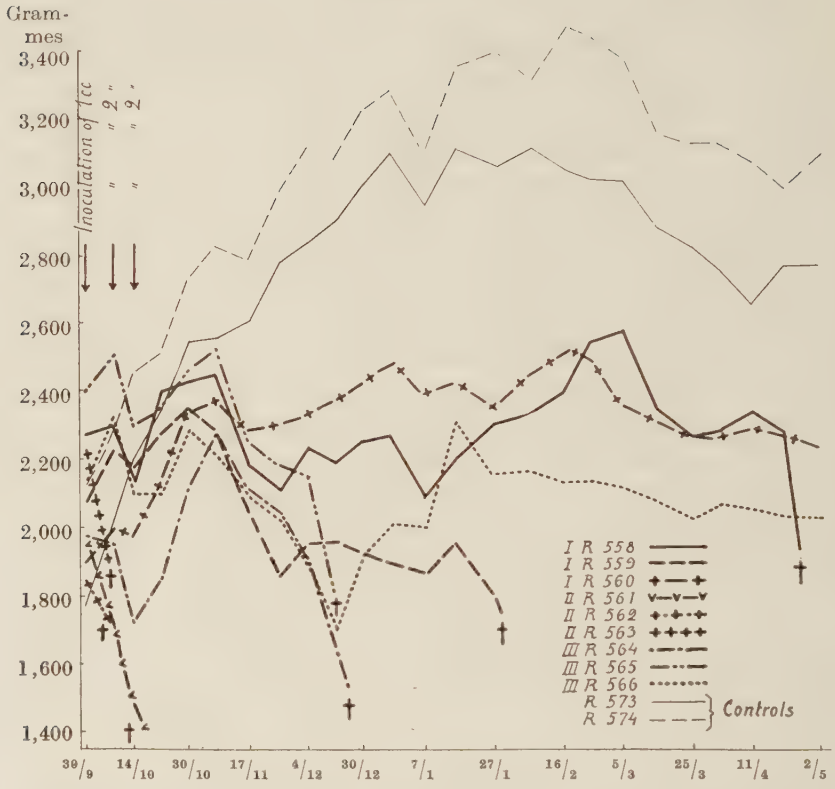
Fifteen rabbits, three for each fungus strain, were inoculated intravenously 3 times with respectively 8, 16, and 22 days' glycerin bouillon cultures of fungus. The first dosis of inoculation was 1 cc, and the 2 ensuing ones 2 cc. The changes in weight of the rabbits, as well as those of the non-inoculated ones, can be followed in diagrams 3 and 4.

Diagram 3 shows that a distinct difference in weight exists between the animals inoculated with the strains I, II, and III, and the controls.

Of the rabbits inoculated with strain I, the first one, R 559, died

[1] According to a statement by Dr. Hedström at the State Veterinary Bacteriological Institute in Stockholm, such subcutaneous abscesses containing gram-negative pasteurella-like rods are very common in rabbit. In the more than 100 cases which he had the opportunity to examine he was neither able to detect acid-fast bacilli in the abscesses nor to place the abscesses in any connection with tuberculous changes (45).

Diagram 3.



cachectic after $2\,^1/_2$ months, but without any macroscopic changes of internal organs. The second, R 558, died intercurrently after about 6 months, and the third, R 560, was killed after $11\,^1/_2$ months. All of them had during their lives shown disturbances of the central nervous system, and the brain of R 560 showed the same microscopic picture as R 552 (spontaneous encephalitis), but in the others only round cell infiltrations or granulomata. The liver of R 560 showed changes that were analogous to those in the brain, though without necrosis or findings of micro-organisms. Both this animal and R 558 had eye infections, and in a vitreous body abscess from the last-mentioned were found plenty of fungus cells.

The rabbits that had been inoculated with strain II died within 17 days after the commencement of the tests, and two of them, R 562 and 563, had only been given one inoculation. In the kid-

neys of these were seen plenty of nodules that held large quantities of fungus cells. In R 561, which 3 days after the last inoculation had shown severe disturbance of balance, were discerned on microscopic examination plenty of abscesses in the brain (Pl. XII, fig. 2), and in these sparse fungus cells (Pl. VII, fig. 4). In the lungs there occurred plenty of bronchogene abscesses, in the liver abscesses, from which on cultivation there was obtained a growth of fungus, and in the kidneys chronic focal nephritis.

Of the rabbits inoculated with strain III, R 556 died after 2 months with paralysis of the back part of the body. It showed granulomatous changes of the lungs, liver and kidneys, as well as pronounced haemosiderosis in the spleen. In smears from the lung and kidney were found sparse fungus cells. The central nervous system showed diffuse round cell infiltrations and granulomata. R 565, which was also fully paralysed in the back part of the body, was killed after 2 months by reason of large, decubital ulcerations in the gluteal and perineal region. Its internal organs showed no appreciable changes. R 566 was killed after $11^1/_2$ months. The internal organs were, apart from a pronounced haemosiderosis in the spleen, without any appreciable changes. Both eyes manifested endophthalmia chronica and dissolution of the lens.

Diagram 4, too, shows a considerable difference in weight between the inoculated animals and the control rabbits. Nevertheless, one of the former, R 568, seems after about 4 months to increase in weight, so that it does in no very great degree differ from one of the control animals.

Two rabbits, R 567 and 569, of those inoculated with strain IV died within $2^1/_2$ months with paresis in the hind legs and reduced weight, but without macroscopic changes in the internal organs. In R 568, which had shown a temporary loss of weight and likewise temporary paresis in the hind legs, there were found in the brain the same changes as in R 552 and 560. In its eye there was present chronic iridocyclitis, with retinal loosening, and in a subretinal abscess were found two small groups of fairly large and slightly clumsy *acid-fast rods* (cf. Pl. VII, fig. 3).

One of the animals inoculated with strain V, R 571, died after four days. There was then found in the left pleural cavity a teaspoonfull of blood-stained exudation, which in smears and in cultivation gave negative findings of micro-organisms. In the liver were

Diagram 4.

seen some miliary nodules, which contained a moderate quantity of fungus cells. R 572 died after 4 months with paralysis in the back-part of the body, double-sided eye infection, and pronounced *cachexy*. Besides bleeding from the lungs, no changes in the internal organs were found. The eyes showed vitreous body abscesses with a large amount of fungus cells, while in the brain only trifling round cell infiltrations were met with. R 570, which had presented moderate paresis in the hind legs, and hyalitis oculi bilateralis, with secondary cataract, was killed after 11 $^1/_2$ months. The rabbit was rather emaciated and showed granulomatous foci in the lungs and liver, fibrosis of the kidneys and moderate haemosiderosis in the spleen. In the eyes was distinguished retinal loosening with an accumulation of pus and a fusion of the edge of the lens, and in the central nervous system diffuse granulomata and fresh degeneration of the pyramidal tract.

Series 3.

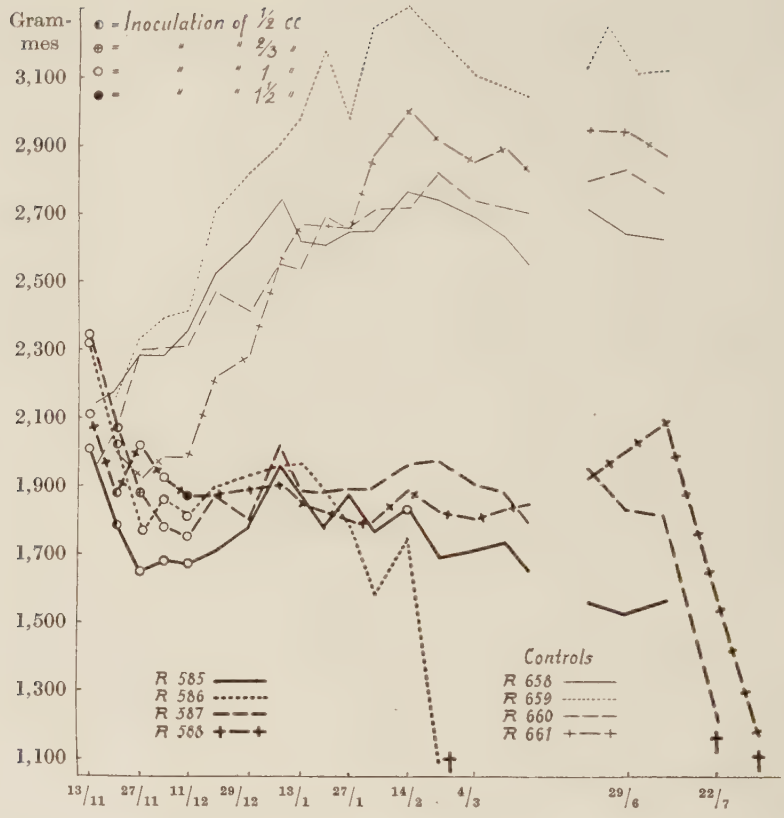
This series was started a few days after the autopsy of R 527, where some findings of acid-fast rods were made in tuberculoid changes. The aim and object here was, in doses of fungus cultures adapted to the general condition of health of the rabbits, to keep their condition down sufficiently for the capacity of the inoculated fungus cultures to bring forth acid-fast rods thus perhaps being facilitated.

As I thought that strain II displayed greater pathogenity, 6 rabbits were inoculated with this and 16 rabbits with the remaining fungus strains, 4 for each of these. In the diagrams 5—9, one for each fungus strain, the doses of inoculation can be read. As the rabbits' general condition of health can largely be judged by their changes in weight, weight curves have, for purposes of comparison, been included in the diagrams for 4 non-inoculated rabbits.

Rabbits inoculated with strain I: Diagram 5 shows that a distinct difference in weight exists between the inoculated animals and the control ones. R 587, 586, and 588, died after respectively $2\,^1/_2$, $7\,^1/_2$, and 8 months, all of them with pronounced *cachexy*. R 587, which had had progressive paresis in the back part of the body, showed in the liver caseous necroses, with a fairly large quantity of fungus cells, while in the central nervous system in the sections nothing certain pathological was met with. In R 586, which had shown the same symptoms as the preceding one, was found a one-sided, strongly hydrocephalus internus, as well as malacic foci and diffuse perivascular glial infiltrations, while in the internal organs, apart from splenic atrophy and trifling changes of the kidneys, nothing appreciably pathological was noticed. In both eyes there existed diffuse changes, with large masses of fungus. R 588 died of coccidiosis, and 585, which had shown some cerebral symptoms, was killed after $7\,^1/_2$ months. In the brain of the latter were found the same changes as those described for R 552.

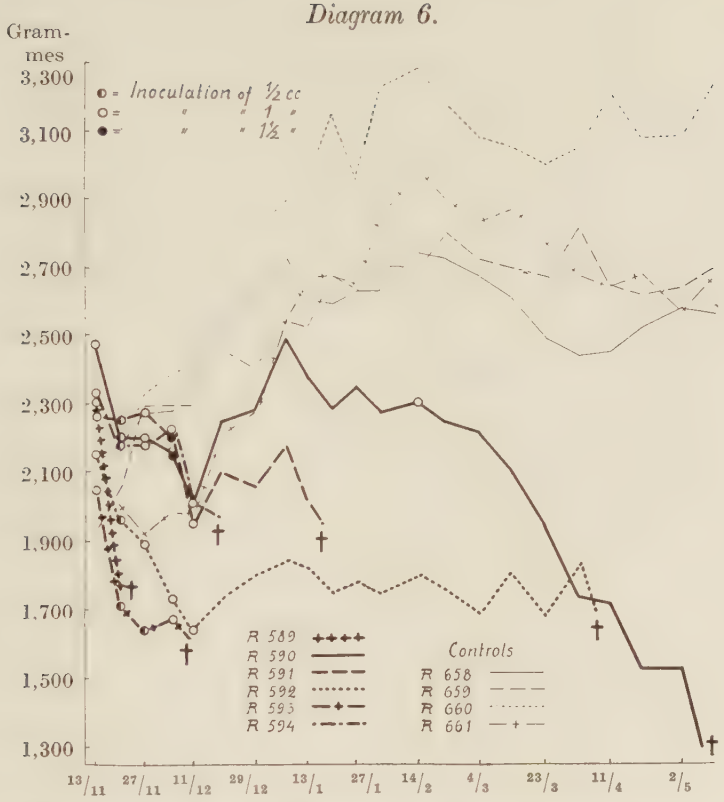
Rabbits inoculated with strain II: Within 6 months all the animals had died. (See diagram 6.) R 589, which died 7 days after the first inoculation, showed in the liver and kidneys nodules the size of a pin's head, consisting of submiliary abscesses, surrounded by more or less distinct granulomatous zones. In the abscesses were found plenty of fungus cells and a few acid-fast, rather short, rods. Cultivation from

Diagram 5.



the kidney on Hohn-medium resulted in a *growth of fungus and acid-fast rods* (see Chapter VIII and Pl. VI, fig. 2). R 590, which had shown paresis in the back part of the body, died *cachectic* after hardly three months. Apart from atrophy of the spleen, with haemosiderosis, the organic changes were slight. R 591 showed after one month symptoms of disturbances of the central nervous system and died a week later, with typical signs of insufficiency of the heart. In the right chamber of the heart there was a pronounced verrucose endocarditis, the verrucose excrescences mostly consisting of large masses of fungus elements (Pl. IX, fig. 1). In the lungs were noticed minor abscesses, and in the liver several necroses (Pl. IX, fig. 2), from which in a cultivation on glycerin bouillon a *growth of fungus and acid-fast rods* was obtained (see chapter VIII). In the central nervous system occurred

Diagram 6.

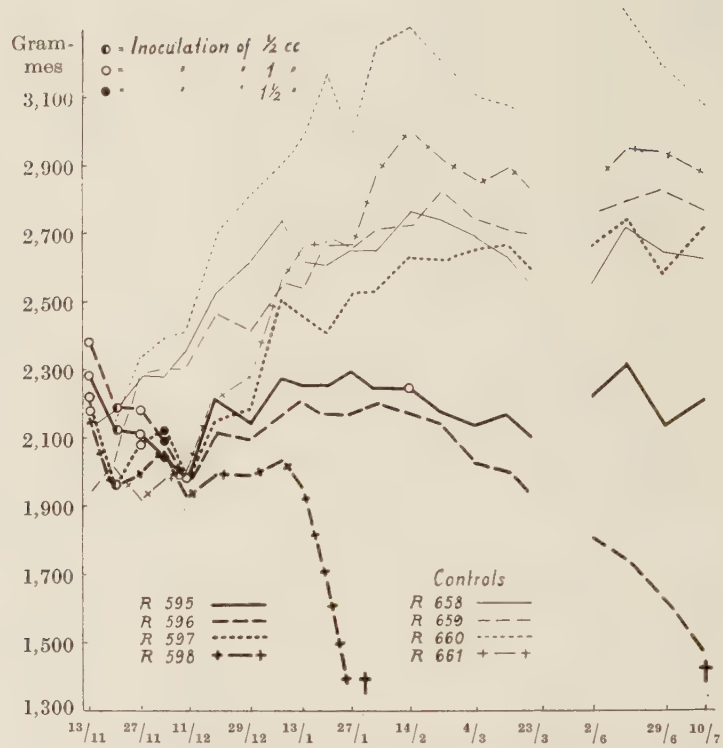


necroses but no fungus cells. R 593, 594, and 592 died, probably intercurrently, after respectively 4 days, 7 days, and 4 months.

Rabbits inoculated with strain III: Diagram 7 shows that one of the inoculated rabbits, R 597, did not differ in weight from the control animals, and another one only slightly.

In R 598 there occurred a week after the inoculation a disturbance of balance and, gradually, progressive paresis, especially in the barrel and hind extremities. The rabbit was inoculated not more than twice and died after about $2\,^1/_2$ months with severe *cachexy*. The spleen presented pronounced haemosiderosis, and the liver minor necroses, which in smears contained plenty of fungus cells that were mostly unstainable. In the brain were met with perivascular round cell infiltrations and solitary glious foci, an abscess the size of a walnut, with a large quantity of fungus cells of the same appearance as those in the liver. R 596, which had shown some loss of strength

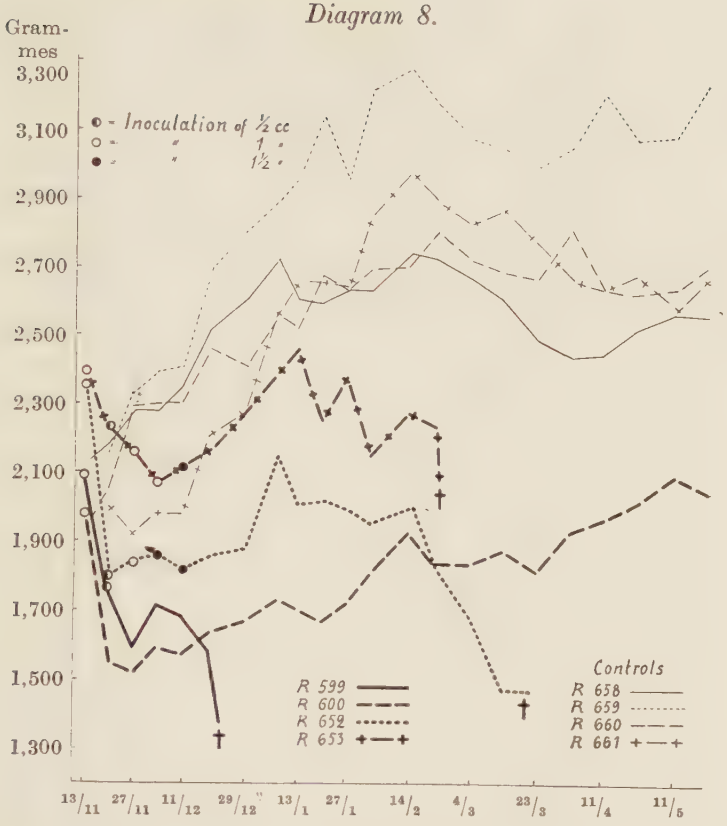
Diagram 7.



in the hind legs, died after 7 months with pronounced *cachexy*. The internal organs showed no appreciable changes. In the central nervous system was found, apart from some perivascular round cell infiltrations, a lump of exudate lying freely in the fourth ventricle, and the bulk of this lump consisted of fungus cells. R 595 and 597 were killed $8\,^1/_2$ months after the commencement of the test. They showed only some uncharacteristic changes in the internal organs. All the three last-mentioned rabbits presented pronounced changes in the eyes in the form of periferal disintegration of the lens and retinal loosening, with subretinal abscesses containing plenty of fungus elements.

Rabbits inoculated with strain IV: In diagram 8 it is seen that there exists a difference in weight between the inoculated and the control animals. The former all display signs of injuries in the central nervous system, and on microscopic examination moderate

Diagram 8.



or fairly pronounced changes there, but without any findings of fungus cells.

R 599, which after the second inoculation proved to be severely affected, was no more inoculated. It died after one month. At the autopsy there was found a large abscess, which issued from the mediastinum and followed the large vessels up into the upper apertura thoracis, after which it passed down along the left half of the thorax and subcutaneously upon the back. The pus contained solitary fungus cells, a gram-negative, non-acid-fast actinomyces-like mycelium, and gram-negative rods. On cultivation from blood and pus there was obtained only a growth of the two last-mentioned organisms. With the exception of severe haemosiderosis in the spleen, no appreciable changes of internal organs were noticeable. R 653 died after $2\,^1/_2$ months with some loss of weight. Postmortal changes

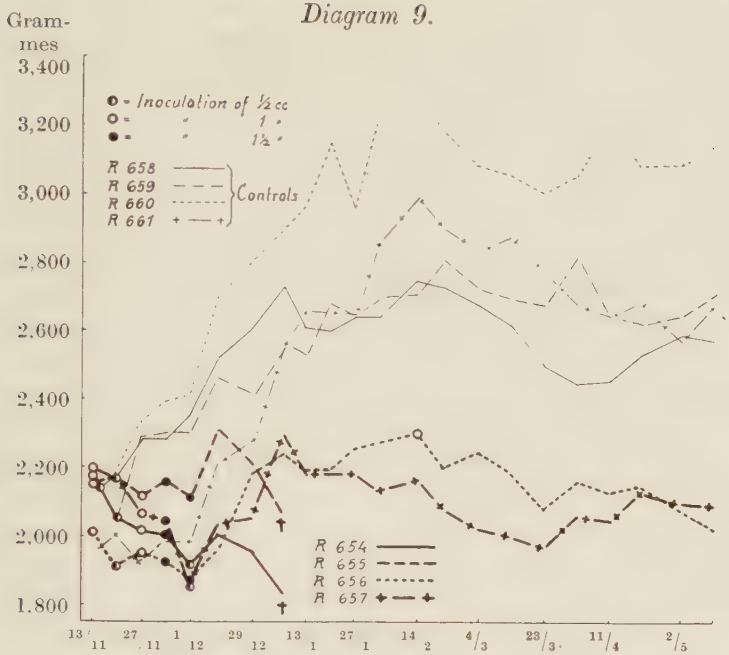
made the findings in the abdominal organs difficult of being judged, but on cultivation from the enlarged spleen no growth was obtained. One eye showed periferal disintegration of the lens. R 652 died *cachectic* after 3 months. In the liver were seen a couple of miliary necroses and in one kidney some submiliary, encapsuled abscesses, in whose centres there were lying some fungus colonies (Pl. XI, fig. 1). In the eyes there existed retinal loosening and abscesses, some of which contained large masses of fungus elements, and one of them furthermore two small groups of *acid-fast, somewhat clumsy, rods* (Pl. VII, fig. 3). R 600, which had been greatly emaciated and had shown disturbance of balance and paresis, improved gradually and increased in weight, so that, when it was killed after $10\,^1/_2$ months, it did not appreciably differ from the control animals. Only moderate changes of internal organs. Both eyes showed retinal loosening and periferal necrosis, as well as disintegration of the lens. In one eye there were also met with in an abscess a couple of fungus cells with a brown self-colour. In the brain were found changes that have been described for R 552.

Rabbits inoculated with strain V: Diagram 9 shows a distinct difference in weight between the control animals and the inoculated ones, two of which however soon died.

R 654 and 655 died both after 3 weeks through external violence (bitten in the scrotum). Their internal organs did not display any appreciable changes. The weight of R 656 and 657 had during the greater part of their lives been stationary, but rose gradually, so that, when the animals were killed about $8\,^1/_2$ months after the commencement of the tests, it did not differ so very much from that of the control animals. Besides a pronounced myocardial fibrosis in R 656, no major changes were discerned of internal organs of the two rabbits, but in the central nervous system there were some diffuse round cell infiltrations or granulomata. R 657 showed changes in the eyes in the form of retinal loosening, and disintegration of the lens and a retinal abscess with a large fungus colony (Pl. XV).

In the case of all the rabbits forming series 3, which had been alive three months after the first inoculation, *tuberculin tests* were carried out. This was done according to Mantoux in one of the rabbits' ear-lobes and with a quantity of tuberculin up to 1 mg. *Negative results* were obtained in all cases.

Diagram 9.



Tests have been carried out in order to ascertain whether exotoxins had assisted in the pronounced diminution of weight which occurred in the majority of the rabbits inoculated with glycerin bouillon cultures of the fungus in question. 10 rabbits were inoculated in this way, 2 for each fungus strain, with 10—30 days old glycerin bouillon cultures after passing through a Zeiss bacteria-proof filter, and by testing the sterility of the filtrate. The animals were inoculated five times, twice with 1.5 cc and three times with 2 cc, but *without loss of weight* or other morbid symptoms occurring.

CHAPTER VII.

Summary of Morbid Symptoms, Pathological Changes, and Bacteriological Findings in the Rabbits Inoculated with the Fungus in Question.

Within 24 hours after intravenous inoculation with cultures of the fungus strains there became manifest in the rabbits a rise in

temperature from normally 37° C—37.6° C up to a maximum of 40.1° C, which after 2 –3 days was succeeded by hypothermy with a minimum of 34.8° C. These temperatures were measured in a series of full-grown, large animals, none of which died of its infection. Two rabbits inoculated with the same quantity of commercial yeast manifested analogous, though small changes of temperature.

Often there occurred a decrease in weight in the rabbits after inoculation with fungus culture[1], which with repeated inoculations was striking, or the weight of the animals kept fairly constant instead of rising in a manner natural for growing animals. During a longer or shorter period an increase in weight might remain absent or a low weight persist before the animal died *cachectic*, which was often the case, R 518, 519, 520, 521, 540, 555, 557, 559, 572, 586, 587, 590, 596, 598, 652, or the weight rose to normal values, R 541, 553, 560, 566, 595, 597. In tests for the purpose of ascertaining whether the decrease in weight was caused by the live fungus cells or their possible endo- and ectotoxins, the two last-mentioned ones in the doses used (see p. 35 and 49) did not show any influence upon the weight of the rabbits.

A certain amount of general atrophy ensued also with the diminution of weight, most easily noticeable in the spleen, which in the cachectic animals might be very small. In these animals there existed also a brittleness of the skeleton, so that in five rabbits an extremity was fractured when the animals were being stretched on the section board, though the utmost care was exercised not to use any force.

During their lives many of the rabbits inoculated with fungus culture manifested signs of infections of the eyes and nervous system, and these symptoms are stated in connexion with the following synopsis of the organic changes. Of these were found partly such as had with certainty been caused by the fungus cells, and partly such as had been due to infections contracted in the stable.

One rabbit, R 591, which died with distinct signs of weakness of the *heart*, showed in the right chamber verrucose, mycotic endocarditis, where the verrucose vegetations mostly consisted of large

[1] In the said synopsis of morbid symptoms and of changes of the organs no animals with certain stable infection (coccidiosis, pasteurellosis, spontaneous encephalitis caused by "Encephalitozoon cuniculi") are included, unless this is expressly stated.

masses of fungus cells (Pl. IX, fig. 1). In another case, R 589, were seen in the myocardium some solitary, granulomatous foci of epithelioid cells, but without any central necrosis. In the surroundings necrotic, calcinated muscle filaments. Twice there was encountered slight, respectively pronounced, myocardial fibrosis, R 588, 656 (the former died of coccidiosis hepatis).

In many cases no changes of the *lungs* resulted, R 540, 541, 553, 555, 557, 560 and several others, or only minor ones in the form of atelectases, haemorrhages, oedema and slight accumulations of round or plasmatic cells, e. g. R 521, 565, 566, 567, 586, 652, 653. Twice there occurred granulomatous foci of epithelioid and round cells, but without necrosis, R 564, 570, of which in the former fungus cells were seen in smears, while in the latter, which showed giant cells in the granulomatous foci, no fungus elements were encountered. In one case, R 527, there occurred granulomata of epithelioid and round cells mixed with uncharacteristic giant cells, as well as focal, caseous necroses containing degenerated fungus cells, acid-fast rods (Pl. VIII, fig. 1) and gram-negative, bipolar rods (see account of this p. 39). Twice there occurred abscesses with fungus cells, R 591 and 593. In the latter animal, which also showed bilateral sero-fibrinous pleuritis, were observed in the surroundings of some of the abscesses uncharacteristic giant cells and in larger or smaller arterial branches purulent thrombotic masses (septic emboli). On cultivation from the lung there was only obtained a growth of short, bipolar, gram-negative rods. In one case, R 518, suspected fungus cells and remnants of such were found in a necrotic focus with central colliquation. Many times there predominated in the lung-picture bronchitis, peribronchitis, and bronchopneumonia, largely certainly caused praeagonally (microscopically fresh changes). In smears from the lungs of two rabbits with bronchopneumonia, R 554 and 561, were found fungus cells.

In the *liver* of some of the rabbits occurred minor round cell infiltrations, e. g. R 520, or submiliary to miliary granulomata of epithelioid and round cells, and in a couple of cases with giant cell formation but without necrosis in the granulomata, R 527, 564, 566, 568, 570, 656, 657. Macroscopically these changes as a rule manifested themselves as small, slightly raised, whitish nodules. In the hepatic granulomata from one of the above-mentioned rabbits, R 527, were found plenty of acid-fast rods, and in another, R 564, solitary

fungus cells. Some of the animals, R 521, 561, 587, 590, 591, 598, 652 (Pl. IX, fig. 2 and Pl. X, fig. 1) showed diffuse necroses in the liver, and in smears from four of them, R 561, 591, 594, 598, were found fungus cells. In the necroses of R 587 no fungus elements were seen, but on the other hand in one of the major bile-ducts necrotic exudations stained and unstained, round and ovoid fungus cells (Pl. X, fig. 2).

The *spleen* was in many cases, particularly in the cachectic animals, strikingly small, and it was possible sometimes to observe microscopically fine atrophy of the parenchyma with close-lying trabeculae. Often there was present a more or less pronounced haemosiderosis in the reticular cells of the pulp, occasionally with giant cell formation. But in two out of four old, non-inoculated rabbits where the spleen was examined microscopically, there was found pronounced haemosiderosis in the pulp, and in one of them, partly in the process of giant cell formation. In the spleen of one of the animals, R 518, inoculated with fungus culture, there occurred miliary epithelioid cellular granulomata, but without giant cells and necrosis, and in another, R 527, quite small granulomatous foci, partly with polynuclear giant cells, but here, too, without necrosis. The foci contained a fairly large quantity of acid-fast rods (Pl. VIII, figs. 2 and 3), which on being cultivated on Hohn-medium grew in pure culture (Pl. XXII, fig. 5). (As regards tests with these rods, see Chapter IX.) In no instance were certain fungus cells met with in the spleen.

In those rabbits that died within the next few days after intravenous inoculation, the *kidneys* seemed often to be strewn with a large quantity of whitish nodules the size of a pin's head and smaller, which contained fungus cells. If the animals died after about 2—3 weeks, the nodules seemed to be still more fibrotic, so that the animals after about 1 month as a rule showed only more or less pronounced cicatrose pits on the surface. An example of the microscopic picture of a kidney from a rabbit that had died one week after inoculation with fungus, is R 589: Both in the marrow and cortex were seen numerous submiliary abscesses surrounded by more or less distinct granulomatous zones without necrosis. In this granulomatous tissue there occurred in several places rudimentary giant cell formations, possibly representing epithelioid proliferations from duct remnants. In smears from the kidney abscesses were seen quite a good many fungus cells and also some solitary

short, acid-fast rods, and in cultivation on Hohn-medium was obtained growth of both fungus and short, acid-fast rods (Pl. VI, fig. 2). — Two further findings of such rods were made in kidney abscesses (in animals that are not included in the rabbits mentioned in Chapters V and VI), once in a rabbit that died 5 days after inoculation with fungus culture, and the second time in a rabbit which had been begun to be inoculated with a mixture of cultures of the five fungus strains (Pl. VI, fig. 3) for the production of immune serum.[1] — In exceptional cases abscesses containing fungus cells could exist quite a long time, 3 months subsequently to the conclusion of the inoculations. R 652 (Pl. XI, fig. 1). In smears from this were seen plenty of fungus cells, some odd ones of which were acid-fast with Hallberg-stain, but the majority not stainable, although the counter-staining was done for a longer period than usual. Even with Gram-staining the fungus cells did not take any stain at all, or only poorly (Pl. VII, figs. 1 and 2). In the kidneys there existed in several cases diffuse, solitary round cell infiltrations and granulomata, e. g. R 520, 541, generally located radially through the cortex and often also the marrow, passing through more or less thick striae of connective tissue with generally gradually decreasing numbers of minor granulomata or round cell infiltrations, R 560, 586, and others (Pl. XII, fig. 1).

No visible changes ever occurred in the *digestive tract* (only a couple of cases have been examined microscopically). In the few animals in which the *suprarenals* were examined these showed, apart from submiliary granulomata and a couple of thin necrotic striae, nothing pathological.

In a large number of cases, 26 out of 42 rabbits inoculated with glycerin bouillon culture (furthermore also in a few inoculated with agar culture), there occurred disturbance of balance or paresis (Pl. XIII). Not once did there occur any signs of injuries in the nervous system in direct connexion with an inoculation. At the earliest, the symptoms in question made their appearance after 3, 6, and 7 days, in respectively R 561, 564 and 597. In the remaining cases the corresponding periods were from 2 weeks to 3 months,

[1] Furthermore, out of 15 rabbits inoculated in the testicles with glycerin bouillon cultures of the fungus strains two, which died after 3 and 5 weeks, respectively, showed local abscesses containing fungus cells and short acid-fast rods. Moreover, in one of the animals was observed a large suspended abscess as well as caseous transformation of the corresponding glands.

in exceptional cases longer, but most commonly 2—6 weeks after the last inoculation. The disturbances consisted in difficulty to maintain the balance, awry head, pareses of varying degree to paralysis in the hind legs, but also some time in the trunk. It was only in exceptional cases that feebleness was manifest in the forelegs or in the musculature of the neck.

Amongst the *brain* and *spinal cord* from 40 animals (35 of these suffering from paralysis or paresis) that had been microscopically examined, mostly both were examined, fungus cells were found in only 3 cases, R 561, 596, 598. But it should be emphasized that no serial sectioning of the central nervous system was carried out, but only 3—6 sections had been taken from the cerebrum, generally just as many from the cerebellum, and 5—10 sections from the medulla oblongata and the medulla spinalis. R 561 displayed both in the cerebrum and in the cerebellum, especially in the cortex cerebri, multiple miliary and minor abscesses with a slight tendency to necrosis, and also in the surroundings considerable astrogliar reaction and perivascular round cell infiltrations (Pl. XII, fig. 2). In some of the abscesses were found fungus cells in typical agglomerations (Pl. VII, fig. 4). In R 598 was observed in one cornu ammonis an abscess the size of a walnut with large quantities of faintly stained, or quite unstained, fungus cells. Moreover, there were seen in the stamgangliae hyperaemia and perivascular round cell infiltrations, as well as in solitary spots small glious foci. The same changes occurred also in one cornu ammonis. In a section from the brain of R 596 was found in the fourth ventricle a lump of exudation hardly the size of a pin's head, the lumen of it being occupied by a fungus colony. In the surroundings was seen a trifling ependymic proliferation. Changes were noticed also here and there in the cerebrum, partial destruction of the pyramidal cell band with glious proliferation, and, in some diffuse spots in the hemispherical marrow, perivascular round cell infiltrations. In 6 cases were found stable-encephalites with well-known etiology, R 693, 552, 560, 569, 585, 600. The changes in the brain consisted here of submiliary to miliary granulomata with central caseous necrosis, which contained plenty of corpuscles that were acid-fast with Hallberg-staining, and were of the same size and outline as those which Levaditi has named Encephalitozoon cuniculi. In these animals there occurred also diffuse granulomata without necrosis and round cell infiltrations. In

all the brains examined occurred, with few exceptions, changes in the form of locally diffused, submiliary, mostly perivascular round cell infiltrations, sometimes also in the meninges, and granulomata as well as minor glious foci. Moreover, occasionally were seen degenerative changes in the central gangliae and pyramidal tracts, as well as diffuse malacic foci. Only once were there found macroscopic changes, viz. severe, unilateral hydrocephalus internus. In the two rabbits which had been inoculated with ordinary commercial yeast, were seen perivascular granulomata. Round cell infiltrations, granulomata, and glious changes were met with also in two out of four brains examined from non-inoculated rabbits, which for over one year had been kept in the stables together with the inoculated ones. Out of the approximately 100 animals which had not been inoculated with live fungus culture, and which I have had an opportunity to observe for nearly two years, there occurred only one case of paresis in the hind legs (one out of a series of 50 rabbits which had been inoculated with dead fungus culture and then inoculated with tubercle bacilli). The central nervous system displayed in this case only uncharacteristic, perivascular round cell infiltrations.

Of the rabbits inoculated with the five fungus strains, macroscopic infections of the *eyes* were found in 12, viz. R 554, 558, 560, 566, 568, 570, 572, 586, 595, 600, 652, 657. In these animals there appeared at the earliest 5 weeks and latest 6 months, in the majority between 2 to 3 months, after the last inoculation unilateral or generally bilateral hyalitis oculi. On making a direct inspection of the eye it was possible to see through the wide pupil, at varying depths in the vitreous body, a light-reflecting tissue consisting of large, reddish-yellow to yellow, rounded formations (more or less vascular protrusion of the retina and hyalitic abscesses?), (Pl. XIV, fig. 1). Some time the vitreous body might appear diffusely greyish with somewhat flocose contents. Simultaneously with these changes there occurred sometimes more or less pronounced hyperaemia in the iris. At the earliest 3 weeks, generally a month or so after the appearance of the changes in the vitreous body, was noticed commencing clouding of the lens (Pl. XIV, figs. 2 and 3). A striking thing was that the animals quite a long time after the beginning of the ocular symptoms, retained some of their sight.

Microscopically there were present pronounced endophthalmitic

changes (such were met with also in one rabbit, R 563, which in life had not shown any distinct infection of the eyes): There were seen retinal and subretinal exudations; subretinal or retinal, often necrotising, abscesses; partial or total loosening of the retina, and proliferation of its glious elements; as well as granulomata, some of which contained plenty of lipoid cells. In the vitreum occurred an increased content of albumin, diffuse agglomerations of cells and large abscesses. In the lens was sometimes seen periferal disintegration or necrosis within the capsule, occasionally with calcination. The chorioidea displayed granulomata, and, sometimes calcinations, and in one case, R 558, composite foci of submiliary round and epithelioid cells, which reminded one of tubercles or changes in sympathetic ophthalmia. Furthermore, there occurred in some eyes also signs of chronic iridocyclitis. In 7 of the rabbits were encountered in one or more abscesses fungus cells, sometimes solitary ones, sometimes in large colonies of round and ovoid cells mostly in the centre and in the periphery elongated with claviform swellings. Some of these colonies were surrounded by fairly broad, hyaline borders (Pl. XV, figs. 1 and 2). In the large colonies only the outside outlines of the fungus cells were noticed to have taken the stain like a nucleiform formation which in the elongated cells continued as a spirally twisted filament. In two of the rabbits, R 568, 652, the former with stable-encephalitis, there were found in abscesses a couple of small groups of fairly short, slightly clumsy acid-fast rods (Pl. VII, fig. 3), in the former case together with solitary fungus cells, and in the latter without fungus elements.

From the subjoined table of the rabbits[1] inoculated with glycerin bouillon culture of the fungus in question is seen partly with which fungus strain they have been inoculated, and partly the number of animals from which culture tests have been carried out, and in which case growth of fungus has been obtained, and partly the number of animals in which fungus elements have been found in the internal organs by culture or in direct preparations, as well as animals in which it has been possible to demonstrate fungus cells in one eye or both, as well as in the central nervous system.

Out of the 42 rabbits in the table, 10 were inoculated with strain II, and with the other strains 8 animals each. Of the 7 animals

[1] The numbers of the rabbits are in brevier, and the Roman figures state the fungus strain inoculated.

Length of life of rabbits after last inoculation	< 10 days	10—30 days	1—2 months	2—3 months	3—6 months	> 6 months
Number of rabbits inoculated with glycerin bouillon cultures	7 R 561[II], 562[II], 563[II], 571[V], 589[II], 593[II], 594[II]	3 R 599[IV], 654[V], 655[V]	3 R 565[III], 567[IV], 591[II]	7 R 529[IV], 564[III], 569[IV], 587[I], 590[II], 598[III], 653[IV]	8 R 526[I], 527[II], 528[III], 530[V], 559[I], 572[V], 592[II], 652[IV]	14 R 558[I], 560[I], 566[III], 568[IV], 570[V], 585[I], 586[I], 588[I], 595[III], 596[III], 597[III], 600[IV], 656[V], 657[V]
Number of rabbits from which culture tests have been made	5 R 561, 562, 571, 589, 593	1 R 599	2 R 567, 591	2 R 598, 653	3 R 527, 530, 592	2 R 558, 586
Number of rabbits from which fungus growth has been obtained	3 R 561, 562, 589		1 R 591			
Number of rabbits from whose internal organs in preparations and by culture fungus elements have been found	7 R 561, 562, 563, 571, 589, 593, 594	1 R 599	1 R 591	4 R 529, 564, 587, 598	2 R 527, 592	
Number of rabbits in which fungus elements have been found in one or both eyes					2 R 572, 652	7 R 558, 586, 595, 596, 597, 600, 657
Number of rabbits in which fungus elements (not Encephalitozoon cuniculi) where found in the central nervous system	1 R 561			1 R 598		1 R 596

that died within 10 days, fungus cells were found in some internal organs of all of them, 6 of them being inoculated with strain II. In culture from organs in five of these cases growth of the fungus was obtained only in 3, and in the other cultivations made, only in one. After a fairly long time had elapsed between inoculation and autopsy, fungus cells were found in the internal organs more and more sparsely, and after 6 months none at all. A long time after the inoculation there were, however, found fungus cells in one or both eyes of 9 rabbits, in 7 of them 6 months afterwards. In the central nervous system fungus cells were found only 3 times.

In the rabbits inoculated with glycerin bouillon culture were also found, though this is not seen from the table, acid-fast rods in kidney abscesses of one, R 589, and in eye-abscesses twice, R 568 and 652, and in lungs, liver, and spleen once, R 527. Growth of both fungus elements and acid-fast rods was obtained from organs of R 589 and 591, but these findings will be dealt with in Chapter VIII. Growth of only acid-fast rods was obtained from liver and spleen of R 527. An account of this will be rendered in Chapter IX.

CHAPTER VIII.

Inoculation of Cultures Containing Fungus Cells as well as Acid-fast Rods into Guinea-pigs and Rabbits.

As described in the second part of chapter IV, acid-fast rods appeared in five glycerin bouillon cultures amongst the fungus cells, viz. two of strain I, one of strain II, and two of strain III. Altogether five guinea-pigs were inoculated with three of these cultures.

One guinea-pig, G-p 238, was inoculated subcutaneously in the right groin with 1.5 cc of the 35 days old fungus culture from strain I, in which only few acid-fast rods were found. A week later an abscess[1] fully the size of a pea was palpated at the spot of inocula-

[1] In subcutaneous inoculation of about 50 guinea-pigs with cultures *merely* containing fungus cells of the strains in question, an abscess the size of from a pea to a valnut is often obtained after 1—2 weeks at the inoculation spot. As a rule, it is resorbed within some little time, at the outside 2 months, or it may fistulate in exceptional cases. The abscess contains fungus cells which occur more and more sparsely the longer the abscess remains. Some time even short, acid-fast rods may be found.

tion, but this had vanished after about 2 weeks. 5 months after the inoculation the animal was killed, and nothing pathological was proved macroscopically. Three guinea-pigs, G-p 319—321, were inoculated subcutaneously in the right groin with respectively 1, 1.5, and 2 cc of the 62 days old culture of strain I, where a large accumulation of acid-fast, granular rods had been found (Pl. IV, fig. 2). Only the animal which was given the largest dose, showed at the inoculation spot after 1 week an abscess twice the size of a pea, which 3 weeks later had disappeared. The guinea-pigs were killed after respectively $2\,^1/_2$, 4, and 9 months, when no macroscopic changes were observed in the internal organs or at the inoculation spot. Unfortunately, no inoculations were made with that culture of strain II in which plenty of acid-fast rods had been found (Pl. III, figs. 4—11. Pl. IV, fig. 1). One guinea-pig, G-p 239, was subcutaneously inoculated in the right groin with 1 cc of the other culture of strain III in which rather plenty of acid-fast rods had been found (Pl. V, figs. 1 and 2). No abscess occurred at the spot of inoculation. The animal died after $2\,^1/_2$ months. The autopsy showed, apart from a moderate loss of weight, nothing pathological.

Inoculations of Cultures of the Strains from Animal Passages.

Two guinea-pigs, G-p 333, 334, and one rabbit, R 687, were inoculated with glycerin bouillon culture originating from renal abscesses from R 589 which had been inoculated with strain II (see Chapter V, diagram 6 as well as Chapter VII) and which contained both fungus elements and solitary acid-fast rods. G-p 333 was given 1 cc intraperitoneally and the other guinea-pig 1.5 cc subcutaneously in the right groin. They were killed after respectively 3 and 8 months and did not show any pathological changes at the autopsy. The rabbit, which had received 1 cc intravenously, displayed after $1\,^1/_2$ month paralysis in the back part of the body and died within 2 months after the inoculation. At the autopsy pronounced *cachexy* was found (loss of weight 690 gr.), but otherwise nothing remarkable. Microscopically was noticed pronounced haemosiderosis in the spleen, and in the brain diffuse malacic and glial foci, while the other organs did not display any, or only trifling, changes. No findings of micro-organisms were made.

From the liver of R 591 inoculated, too, with strain II (see the above-mentioned Chapters), a glycerin bouillon culture had been made. This culture showed fungus after 24 hours at 37° C, and within 7 days also plenty of acid-fast rods. In smears taken after 7 to 14 days' growth were found amongst the fungus elements the acid-fast rods largely arranged in bundles, which in size and outline showed similiarity with ovoid fungus cells (Pl. V. figs. 4—7). — These figures agree fully with those of Reenstierna (103) from 1912. — From a non-acid-fast mycelial branch seems to issue a formation of acid-fast rods (Pl. V, fig. 8). Amongst the fungus cells there occurred acid-fast rods partly lying close together, forming balls (Pl. V, figs. 9 and 10), and partly in large irregular agglomerations (Pl. VI, fig. 1). Even acid-fast fungus elements were found in Ziehl-Neelsen stained preparations (Pl. V, figs. 11 and 12). In an agglomeration of non-acid-fast fungus cells of ordinary size was noticed a larger acid-fast ovoid cell, ostensibly consisting of closely packed, acid-fast elements. At one end of this formation there projected a rod-shaped "handle" (Pl. V, fig. 12).

Three guinea-pigs, G-p 329—331, and 4 rabbits, R 683—686, were inoculated with the said culture. G-p 329 was given subcutaneously in the right groin 1.5 cc of the 7 days old culture, and G-p 330 and 331 intraperitoneally respectively 1.5 and 1 cc of the 11 days old culture. After 1 month G-p 329 had a healed fistula in the right groin (the animals were not observed in the interval). G-p 331 was killed after about 3 months and the remaining ones after 8 months. None of the animals displayed any appreciable changes of internal organs. Of the rabbits, R 683, which was inoculated intravenously with 1.5 cc of the 7 days old culture, manifested after 4 days paresis of the hind legs and died one day later. In the lungs were noticed haemorrhagic foci, and in the kidneys a remarkably large quantity of white nodules the size of a pin's head, which contained a large quantity of fungus cells, as well as solitary, short, clumsy, acid-fast rods. On making a culture from the kidney abscesses on glycerin bouillon, only growth of fungus was obtained. A microscopic examination was made only of the central nervous system, which displayed diffuse abscesses (Pl. XII, fig. 3), containing numerous fungus cells, some of which, lying in agglomerations, were plentifully provided with granules (Pl. VII, fig. 5), which imparted to the cells a certain likeness to the "cones" descri-

bed by Hallberg (43). R 684, which was inoculated with 2 cc of the 9 days old culture, died after 7 days. The rabbit had then lost 760 gr in weight. The lungs and kidneys displayed abscesses (Pl. XI, fig. 2), which contained plenty of fungus cells, while no acid-fast rods could be found. On making a culture from the kidney abscesses on glycerin bouillon, a growth of fungus was obtained, and in a smear after 6 days at 37° C acid-fast, fairly short rods lying in the form of a tuft, could be seen. R 685 was inoculated with 0.5 cc and 0.75 cc of the glycerin bouillon culture originating from R 591, 11 respectively 21 days old. 6 weeks after the last inoculation, when the rabbit had lost 850 gr of weight, there occurred moderate hyalitis oculi bilateralis. 9 months after the last inoculation, when the animal was killed, it showed cataracta oculi bilateralis. The rabbit was then well nourished and did not manifest any appreciable changes of internal organs. In both eyes were noticed pronounced changes, and in one eye could be seen in an abscess plenty of fungus cells surrounded by a hyaline border. R 686 which was inoculated with 1 cc of the 11 days culture, died after 18 days. The rabbit was lean, loss of weight 1,540 gr. It showed focal, purulent nephritis with interstitial, strong fibrosis, and beneath the surface of the kidney cortex solitary, small abscesses. In the abscesses were discerned some highly suspected fungus cells, but on cultivating in glycerin bouillon and on Hohn-medium no growth was obtained. In the mesenterium were found a few small cysts, which contained parasites, certainly belonging to the intestinal worms.

CHAPTER IX.

Passages in Rabbits and Guinea-pigs with Material (crushed organs and culture) from R 527, which had been Inoculated with Fungus Strain II.

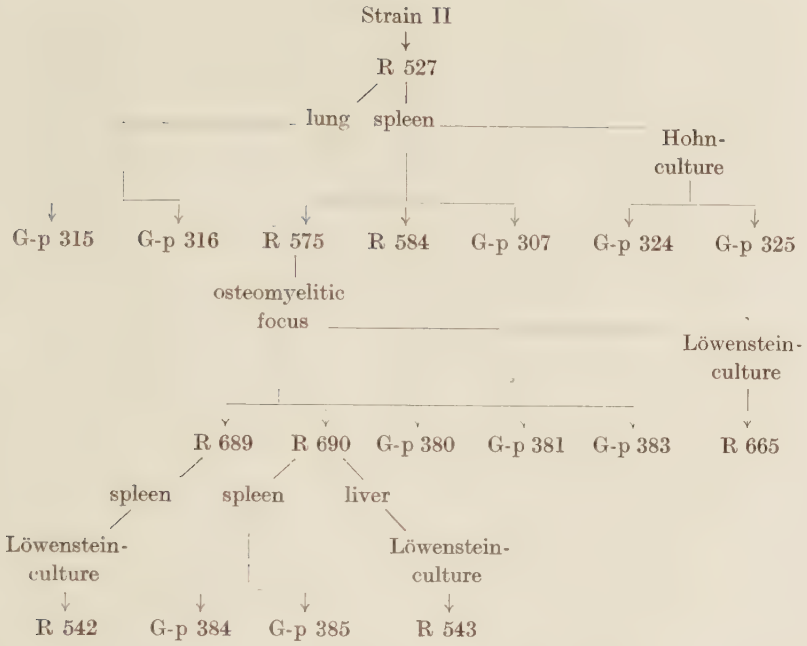
One of the rabbits inoculated with glycerin bouillon cultures from the fungus strains, R 527, as described in Chapter VI, manifested in the lungs, liver and spleen changes which reminded one of tuberculosis, and consisted in the lungs microscopically of granulomata of

epithelioid cells or mixed with giant cells, and in the centre caseous necroses, which latter were absent in the liver and spleen (Pl. XVI, fig. 1). Acid-fast rods were met with in moderate quantities in the three said organs (Pl. VIII, figs. 1—3) and in the lungs, furthermore, short, gram-negative, non-acid-fast rods. This was one of eight rabbits whereof five had been inoculated, which had contracted a stable-infection in the form of subcutaneous abscesses with short, gram-negative, non-acid-fast rods. When making a culture from the liver and spleen of R 527 on Hohn-medium, a growth was obtained in fourteen days of rounded, moistly glittering, greyish-white colonies fully the size of a pin's head, which after a few days at living-room temperature assumed an almost chrome-yellow colour (Pl. XXII, fig. 5). After 3—4 generations the colonies, after the cultures' standing in living-room temperature for some days, did not manifest the same intensely yellow colour as the first generation, but they were more sulphur-yellow. No other micro-organisms than the *acid-fast rods* could ever be proved in the cultures. In cultures of these on gelatine and Hohn-medium at living-room temperature, on slanted agar and blood-agar plate at 37° C, and on Hohn-medium at 45° C, no growth was obtained.

Starting from bits of the lungs and spleen of R 527, and cultures from the last-mentioned, inoculations into rabbits and guinea-pigs were carried out, from which then in their turn fresh inoculations and cultures were made, as is seen from the subjoined synopsis.

A bit of one of the lungs of R 527 the size of a brown bean was crushed and emulsioned in 10 cc NaCl-solution, after which two guinea-pigs, G-p 315 and 316, were inoculated subcutaneously in the right groin with 1 cc and 1.5 cc respectively. The animals were killed after respectively $2\,^1/_2$ and $10\,^1/_2$ months. The former displayed chronic productive splenitis, and also the other one non-specific changes of the spleen, while the other organs did not manifest anything pathological.

A piece of the spleen about the size of a brown bean from R 527 was crushed and emulsioned in 10 cc NaCl-solution, whereafter 1.5 cc respectively 1 cc was inoculated intravenously into R 575 and 584, and subcutaneously into the right groin of G-p 307. R 575 increased in weight during the next 3 months, after which it grew thinner, the loss in weight then being 1,170 gr. 5 months after the inoculation there was observed an increasing protrusio bulbi sinister, and 1 month



later weakness in the hind legs. When the rabbit died, 6 months after the inoculation, it was extremely *cachectic*. Outside the left temporal bone was noticed a caseous focus the size of an almond, which pushed the left eye forward, and microscopically proved to be a partly calcinated, caseous abscess, *very strongly reminiscent of an old, tuberculous, caseous focus*. It was surrounded by a fibrous capsule with *epithelioid cell granulomata with caseous necroses, greatly resembling tubercles* (Pl. XVII, fig. 2). On both elbows and knees, as well as in the left joint of the foot, were noticed large, knotty, caseous layers (Pl. XVI, figs. 3 and 4), and also near the thoracal part of columna vertebralis a caseous focus the size of a pea, all of them being found microscopically to be necrotic osteomyelitic foci surrounded by a border of epithelioid cells. In all the foci there occurred *acid-fast bacilli of Koch's type*, partly diffuse and partly in agglomerations (Pl. VIII, fig. 5). On the apices of the lungs were observed abscesses and otherwise small, whitish foci, which microscopically were found to consist of non-necrotic granulomata of epithelioid and round cells, and contained sparse *acid-fast rods*. The normal-sized spleen displayed macroscopically enormous haemosiderosis and diffuse *epithelioid*

cell granulomata with numerous rudimentary giant cells, as well as a central necrosis with calcination and large quantities of acid-fast rods. The kidneys manifested on the surface hyaline, cicatrose pits, and in the parenchyma some whitish nodules. Microscopically, there were seen cicatrose striae through the cortex, as well as *fresh cortical granulomata of masses of epithelioid cells and central caseous necroses with acid-fast bacilli. Great similarity with tuberculosis.* The ileum and caecum displayed on the surface plenty of yellow foci (Pl. XVI, fig. 2), consisting of granulomata lying in the submucosa with a moderate quantity of *acid-fast rods.* In the brain was found, apart from small, glious and insignificant perivascular granulomata, an older abscess with central, caseous necrosis containing *enormous quantities of acid-fast rods*, lying partly in bundles and partly in balls (Pl. VIII, fig. 4). R 584 was killed after 3 months, being then well-nourished. In the right lung was observed a largish, hyaline part which microscopically was seen to consist of an epithelioid cell-like proliferation of the alveolar walls. The spleen was of normal size, with a few white nodules the size of a pin's head (Pl. XVIII, fig. 1 a), which microscopically was found to consist of *epithelioid cell granulomata with numerous rudimentary giant cell formations*, and one and another granuloma *commencing to turn into a caseous necrosis.* Rather plenty ot *acid-fast rods.* G-p 307, which after 3 weeks at the spot of inoculation displayed a subcutaneous abscess the size of a hazel-nut, was killed after a further 3 weeks. No changes had taken place in the internal organs. In the right groin was noticed a subcutaneous abscess, which contained rather sparse *acid-fast rods*, and was surrounded by a membrane with the appearance of granulomatous tissue rich in cells, with epithelioid cell granulomata in one spot, but without giant cells or necrosis.

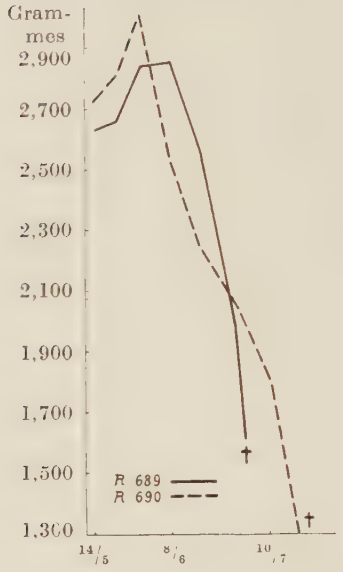
Two guinea-pigs, G-p 324 and 325, were inoculated subcutaneously in the right groin with 0.5 mg respectively 0.25 mg of the 57 days old Hohn-culture from the spleen of R 527. Both displayed after 10 days in the groin an abscess the size of a hazel-nut, which after quite 2 weeks fistulated. G-p 324 was killed after 9 months, and no pathological changes were found. G-p 325 was killed after $3\,^1/_2$ months. In the right groin were observed two abscesses the size of a pea, in places necrotic, and surrounded by a narrow border of epithelioid cells and containing plenty of *acid-fast rods*, separately and in agglomerations, some of them under phagocytosis (Pl. VII, figs. 6, 7). On

the right side of the arteria iliaca dexter was found a lymphatic gland the size of a hazel-nut kernel, which contained *epithelioid cell granulomata with central abscess formation*, but without necrosis.

From an osteomyeltic focus in the region of the left knee-joint of R 575 and the spleen of R 584, Löwenstein-cultures were made. In both growth of sulphur-yellow, moist, semiglobular colonies of *acid-fast rods* up to twice the size of a pin's head was obtained after 14 days (Pl. XXII, fig. 6). The third and following generations of the culture from R 575 showed, apart from the above-mentioned colonies, also larger, unevenly convex ones with pits in the centre (Pl. XXII, fig. 7).

A dab the size of a pea from the osteomyelitic focus in the left joint of the knee of R 575 was emulsioned in 10 cc NaCl-solution, after which two rabbits, R 689, 690, were inoculated with this intravenously with respectively 1 cc and 1.5 cc, and three guinea-pigs, G-p 380, 381, 383, the first-mentioned intraperitoneally with 1 cc and the other two subcutaneously in the right groin with respectively 1 cc and 1.5 cc. The two rabbits increased to start with in weight, after which there was a severe drop in weight. The animals died after respectively $1\,^1/_2$ and 2 months. (See weight curve below.) R 689 showed severe *cachexy* and a *greatly increased spleen* (10 cm long) pervaded by nodules the size of a pin's head (Pl. XVIII, fig. 1), and microscopically pervaded by large and partly confluent *caseous necroses*, surrounded by thinner or broader *epithelioid cell borders*. Here and there *typical Langhans' giant cells*. Histologically, the changes were on the whole *identical with tuberculous changes* (Pl. XIX). The necroses were pervaded by *enormous quantities of acid-fast bacilli*, especially in the periphery, where they were lying partly diffuse and partly in large balls, and also partly in macrophagi and in the Langhans' giant cells (Pl. VIII, fig. 8). Nodules also occurred in the lungs, liver, and kidneys, though not so plentifully. Microscopically, the changes manifested themselves chiefly as *typical miliary tubercles*, having in the lungs moderate and in the liver plenty of *acid-fast bacilli*, diffuse and in bundles or balls. The nodules in the kidneys consisted of granulomata without caseous necrosis or giant cells, partly composed of epithelioid cells. Here and there *acid-fast rods*. R 690 showed at the autopsy severe *cachexy*. The lungs were pervaded by major or minor caseous foci (Pl. XVIII, fig. 2), and microscopically they displayed as a rule in the parenchyma, especially

Diagram 10.



below the pleural surface, *miliary and major tubercles*, with *caseous necroses* and purulent mixtures, and also in the surroundings diffuse *Langhans' giant cells* (Pl. XX). A moderate quantity of *acid-fast rods*. In the liver and normally sized spleen were observed plenty of nodules the size of a pin's head (Pl. XVIII, fig. 2), and microscopically *numerous miliary tubercles with caseous necroses and Langhans' giant cells* (Pl. XXI, fig. 1), and plenty of *acid-fast rods*, singly or in accumulations. Similar foci, though without typical giant cells, occurred also in the kidneys. G-p 380 died after 5 months and was then lean but manifested no pathological changes. G-p 381 lived in perfect health for another 7 months after the inoculation. In G-p 383 was after 14 days observed at the spot of injection a subcutaneous abscess the size of a hazel-nut, which was still there when the animal died about 5 months later. The guinea-pig was then lean. The abscess in the groin, which contained yellowish-white pus and fairly sparse *acid-fast rods*, was surrounded by a membrane with numerous epithelioid cells. A slightly enlarged lymph gland near the arteria iliaca displayed slight, chronic adenitis. No specific changes were observed in the internal organs.

0.2 mg of a 36 days old Löwenstein-culture (third generation)

from an osteomyelitic focus of R 575 was inoculated intravenously into a rabbit, R 665, which died after 3 weeks. Its lungs, liver, and enlarged spleen presented a fine-grained surface but no macroscopic foci. There were observed plenty of *epithelioid cell granulomata*, without necrosis. Only in the liver were found here and there rudimentary giant cell formations. Particularly in the spleen and liver occurred *remarkably plenty of acid-fast rods*, largely in bundles and balls. No specific changes were noticed in the kidneys.

From the spleen of R 689, from the liver of R 690, and from the inguinal abscess of G-p 383 Löwenstein-cultures were made, where the acid-fast rods grew after 14 days, and, in so far as the two first-mentioned were concerned, with colonies of the same appearance as described for the third generation of R 575 (Pl. XXII, fig. 8). The cultures from G-p 383 manifested, however, a different appearance. After a little more than 14 days the colonies were scarcely the size of a pin's head, greyish-white and not so moist (Pl. XXII, fig. 9). After another 2 weeks they were somewhat larger. Although the full-grown cultures were then kept at living-room temperature, the colonies assumed only a slightly yellowish tinge. Microscopically, it was impossible to distinguish the cultures. All of them contained only *acid-fast rods*, somewhat shorter than those observed in the organs in question.

0.2 mg of the 36 days old Löwenstein-culture from R 689 was inoculated intravenously into a rabbit, R 542. The latter died after 16 days. Apart from an enlarged spleen no macroscopic changes could be seen. The lungs manifested intimated bronchopneumonic infiltrations with a moderate amount of *acid-fast rods*. The liver was pervaded by granulomata with solitary minor giant cells, but no necrosis (Pl. XXI, fig. 2). Plenty of *acid-fast rods*, largely lying in bundles and balls. In the spleen, too, were observed granulomatous foci, indistinctly outlined, here and there with intimated necroses. The same findings of bacilli as in the liver. In one kidney was found a submiliary, granulomatous focus of epithelioid cells, surrounded by round cells. Some groups of *acid-fast rods* were encountered, inter alia in a couple of glomeruli.

A bit the size of a pea of the spleen from R 690 was crushed and emulsioned in an NaCl-solution, and inoculated subcutaneously into the right groin of two guinea-pigs each, G-p 384 and 385. In both there was manifested after about 2 weeks at the spot of injection an

abscess the size of a walnut and hazel-nut, respectively, which in the case of G-p 385 fistulated a week later. 3 months after the inoculation, when G-p 384 was being treated on its back with barium sulphide for the purpose of getting a surface free from hair for tuberculin tests, the guinea-pig died within 14 hours. The inguinal abscess contained fairly plentiful *acid-fast rods*. In a lymph gland near the arteria iliaca dexter were observed numerous submiliary epithelioid cell granulomata without caseous necroses, and without acid-fast rods. Only trifling microscopic changes were observed in the internal organs. G-p 385 lived in perfect health for another 5 months after the inoculation.

0.1 mg of the 36 days old Löwenstein-culture from the liver of R 690 was inoculated intravenously into a rabbit, R 543, which died after 5 weeks. The spleen was enlarged, while otherwise no appreciable macroscopic changes were manifest. Microscopically, there were observed in the lungs, liver and spleen *numerous miliary and submiliary tubercles*. Histologically, *great similarity with tuberculosis*. A moderate quantity of *acid-fast rods* was found in the said organs.

Tuberculin tests were carried out in five of the animals accounted for in this chapter, viz. R 665, 543 and G-P 380, 381, and 385. The rabbits were tested 16 days after the inoculation, G-p 380 and 381 after 5 months, and G-p 381 after 3 months. The tests with the rabbits were carried out intracutaneously in the shells of the ears, up to 1 mg of human tuberculin and 0.1 cc non-diluted aviary tuberculin being used, and on the guinea-pigs intracutaneously on the back, after the hair had been removed there with barium sulphide. In the tests with the guinea-pigs was used, apart from the said tuberculin, also a mixture of human and bovin tuberculin up to 1 mg. In no case was a typical positive tuberculin reaction obtained. On the sites for several of the tuberculin test-spots necroses were, however, obtained in the guinea-pigs two days after the injection, which was not seen in the controls with NaCl-solution. In order to be able to exclude the ability of the acid-fast rods to set up tuberculous allergy it will, however, be necessary to make more tests than have here been carried out.

Compared with R 527, the organic changes in the rabbits inoculated with acid-fast rods in culture, or the rabbits inoculated with organic material containing acid-fast rods, have *grown more and more resembling tuberculosis*, if we ignore R 665 and 542, which died respec-

tively 21 and 16 days after inoculation, which may possibly have been the reason why no caseous tubercles had time to develop. In all the changed organs were encountered *acid-fast rods of Koch's type.* They do, however, not appear to have been pathogenic for guinea-pigs, where with subcutaneous inoculation only an abscess occurred at the spot of inoculation, and besides in two cases changes in a corresponding lymph gland, in one of them with abscess formation with acid-fast rods.

CHAPTER X.

Discussion.

The initial material for the present investigation consisted of five fungus strains, isolated from sanatorium patients who were either being treated under the diagnosis of pulmonary tuberculosis, cases III and V, or had been under observation for suspected such, cases I, II, and IV. The five fungus strains agreed with each other in appearance, and with those that had been isolated by Reenstierna (103) from tuberculous material in 1912.

When the fungus had been cultivated over twelve months on ordinary agar tubes, single cell cultures were established of each strain by means of a micromanipulator, after which cultivation on ordinary slanted agar was made for 10 generations. It must therefore be considered out of the question that tubercle bacilli should after that have been present in the cultures when subsequent animal tests were carried out with the same, or with cultivations on media favourable for tubercle bacilli. Since we have not as yet any established method of classification for the fungi imperfecti, i. e. the yeast-like filamentous fungi, in which it has been impossible to demonstrate any sexual reproduction, I have not tried to classify my fungus-strains. The strains showed, irrespective of their varying capacity to produce acid-fast rods, varying but mutually the same cultural, tinctorial, and biochemical properties. On ordinary slanted agar the fungus grew in colonies which corresponded to the R- and S-form of the bacteria, of which the latter was the one most commonly occurring. Such dissociation has been described in yeast-

like fungi during the years 1937—1938 by Besta (12), Castellani (22), and Pinkerton (92). Two types of growth could be distinguished also in glycerin bouillon cultures, partly as cloudy, stringy masses, which might grow along the sides of the flask and out over the surface of the medium in the form of a soft pellicle, partly, though less commonly, as a granular growth only on the bottom of the flask. The stainability of the fungus elements varied to a high degree, according to the age of the culture, so that the acid-fastness with Hallberg-stain, and also gradually the general stainability, diminished with gradually increasing age of the culture. The ability of the fungus-strains to disintegrate certain carbohydrates during the development of gas, was investigated, when this ability proved capable of changing in the course of six months, something that Castellani showed possible to take place.

In five glycerin bouillon cultures from three of the fungus strains originating from single cells there appeared amongst the fungus cells acid-fast rods. In view of these findings one may ask how the acid-fast rods could be generated. No definite answer can be given to this question, but if all the observations made of acid-fast rods emanating from fungus cells are summarized, one can get a certain idea of the course of development. It is therefore necessary to compare my findings with previously made ones, viz. by Reenstierna and by Gullberg.

Reenstierna (103) observed in 1912, as mentioned in the historical survey, in two glycerin bouillon cultures made from single cells of a yeast-like fungus originating from tuberculous material, how the acid-fast rods, precisely like Koch's bacilli, which had appeared could lie ordered in formations that presented the same size and contour as the surrounding ovoid fungus cells. He considered that the acid-fast rods were formed in the fungus cells, which then disintegrated and evacuated their content. Some of his illustrations reproduce both ovoid, round, and elongated fungus cells in states of disintegration. That the acid-fast cells could develop in the manner stated, is apparent also from Gullberg's researches in 1933—1935 (41). An interesting formation, which probably constitutes the preliminary stage of such an ovoid formation as that described by Reenstierna, was observed by me in a glycerin bouillon culture originating from the liver of a rabbit, R 591, which had been inoculated intravenously five times with glycerin

bouillon culture of strain II (Pl. 4, fig. 12), viz. a large acid-fast cell, ostensibly consisting of closely packed acid-fast elements. In the upper part of this formation there projected a rod-shaped "handle". Furthermore, there were also found in the same culture acid-fast rods lying like ovoid formations (Pl. V, figs. 4—6), as well as conglobations of acid-fast rods (Pl. V, fig. 10), probably originated through propagation of the original rods lying in ovoid formations.

In two figures (Pl. IV, figs. 5 and 6) taken from Gullberg's work one imagines to see a course of development of the acid-fast rods slightly differing from the aforesaid. Fig. 5 illustrates between long fungus cells a non-acid-fast, amorphous mass with granules and very short rods, some of which are acid-fast. Fig. 6 shows a large, round formation, whose periphery consists of a wreath of ovoid fungus cells, while its centre is occupied by a net-work of blue filaments (Ziehl-Neelsen stain), in which there are acid-fast rods. These pictures seem to me to illustrate two stages of the formation of acid-fast rods.

Furthermore, I fancied several times that I had found in glycerin bouillon cultures of the fungus strains II and III some fine, unstained, mycelial filaments. Also stainable, shorter and longer, filiform elements have been seen. Thus Pl. III, fig. 5, illustrates rod-shaped ones, with acid-fast granules at the ends, while fig. 6 of the same plate shows filiform elements, of which some are acid-fast, whereas fig. 7 shows only acid-fast ones. Pl. IV, fig. 1, shows a ragged bundle of acid-fast rods and filiform elements, of which one of the latter seems to issue from a round fungus cell. The said filiform elements must have developed from the fungus cells, and the short, rod-shaped ones may have originated through fragmentation of the longer ones.

By way of summary one might be able to say that the acid-fast rods no doubt originate from an amorphous mass in the fungus cells, which via non-acid-fast granules and filaments differentiate into acid-fast elements. Sometimes, the outlines of the fungus cells seem to disappear first after this development, sometimes the fungus cells seem to disintegrate before its contents have been differentiated into acid-fast elements.

This must, of course, only be considered as hypotheses for a couple of probably several possibilities for the formation of the acid-fast rods, which is certainly of a very complicated nature.

Intravenous inoculation into rabbits of agar cultures of the fungus strains produced a change in the body temperature, analogous to that shown by Ostrowsky (87) with fungus isolated from thrush. In my animal tests were employed, as far as ever possible, not fully grown rabbits, which, with few exceptions, after the inoculations did not display the same rising weight-curve as the non-inoculated ones. Several of the animals died cachectic, something which had previously been pointed out by some authors, Reenstierna (103), Pijper (91), Gullberg (41), and Krauspe (59) after inoculation with yeast-like fungus. Often there was manifest distinct splenitic atrophy, and the brittleness of the skeleton was striking.

The inflammatory changes of the internal organs were more pronounced, the sooner the animals died after inoculation, but not so generally disseminated as has mostly been described after inoculation with fungus of yeast type. This may be explained by lowered virulence in the fungus strains on account of prolonged cultivation on media — more than 12 months.

Inflammatory changes in the form of abscesses, round-cell infiltrations and granulomata were found mostly in the kidneys, and next to them in the liver, the lungs, the heart, and the suprarenals, and furthermore also degenerative changes, especially hepatic necroses. Only in one rabbit, R 527, were tuberculoid changes obtained in the lungs, liver, and spleen, which in the lungs consisted of granulomata of epithelioid cells mixed with giant cells up to the size of a pea, and also here and there in the granulomata caseous necroses, but in the liver and spleen of small granulomatous foci without necrosis. In the liver and spleen were met with acid-fast rods, which on being cultivated grew in pure culture, while similar ones in the lungs occurred together with gram-negative, non-acid-fast, pasteurella-like rods. I shall revert later to attempts at explaining the origin of these foci.

In a large number of cases, 26 out of 42 inoculated with glycerin bouillon culture (furthermore also in a few inoculated with agar culture), there occurred disturbance of balance or paresis. Here the symptoms have possibly been obtained by co-operation between the fungus cells and other micro-organisms, inasmuch as the neurological symptoms only rarely occurred in the non-inoculated rabbits in the stable, while histological changes of the same nature, small granulomata, and in general perivascular infiltrations, could be

proved to exist in practically all rabbits, both inoculated and non-inoculated ones. In six cases there occurred furthermore changes in which Encephalitozoon cuniculi could be proved. Only in three cases were yeast-like fungi observed in the central nervous system.

In 12 rabbits occurred macroscopic ocular changes in the form of hyalitis, in the majority of cases with secondary cataract. Microscopically there was present considerable retinal destruction, with abscesses as well as necroses of the periphery of the lens.

In altogether 24 rabbits out of the 42 inoculated with glycerin bouillon culture of the fungus strains, fungus cells were encountered somewhere. Fungus elements were also found in some rabbits which had been given injections of agar culture. In 15 cases the fungus cells could be proved in the pectoral or abdominal organs, while a growth of the fungus in question was obtained by culture in only 4 cases. Of these animals, from which a growth of fungus was obtained, 3 had died within 10 days. Although the material is too scanty to enable safe conclusions to be drawn, the results nevertheless point to the possibility of finding fungus cells in the pectoral and abdominal organs, the same as the obtaining of growth by cultivation from here is greater a short time after inoculation than later. Of the 15 animals from which cultures were established, there were found in smears or sections fungus cells in 9, but cultures of these were only obtained in 4 cases. That a growth of fungus failed to take place, although fungus cells could be proved in smears, has been pointed out by Reenstierna, who considers this perhaps to be due to the fungus cells being in a state of disintegration.

Although the fungus cells could not be proved as a rule after about a month in the pectoral and abdominal organs, it was nevertheless possible, after a long time, to observe them elsewhere. After 6 months there was found a largish accumulation of fungus cells in the fourth ventricle of the brain of one rabbit, and after the same period in the eyes of 7 animals. A striking feature is that in so relatively many cases of ocular changes fungus cells could be proved after such a long time as six months. The explanation of this is probably that the content of antibodies in the vitreum in ocular infections, which Kuffler (61) has shown in 1912, is very small. He found with a content of antibodies in the blood of 1:10 000 that the same was 1:10 in the vitreum.

Acid-fast rods were observed in 4 animals out of those included in the series, in lungs, liver, and spleen of one, R 527 (whose organic changes have previously been mentioned in the discussion), in nephritic abscesses of one, R 589, and in ocular abscesses of two, R 568 and 652. Acid-fast rods were furthermore found in abscesses of another 4 rabbits, although not included in the animal series dealt with, but mentioned in Chapter VII. Although thus acid-fast rods could be proved in altogether 8 rabbits, organic changes caused by the acid-fast rods appear to have occurred only in one, R 527. The general condition of this animal, which suffered from an intercurrent affection, caused by gram-negative, non-acid-fast, pasteurella-like rods (see account of this in Chapter VI) was greatly impaired, which may explain the fact that the acid-fast rods were able to overcome the animal's power of resistance; and become pathogenic. There did not exist any basis for assuming that these were derived from the intercurrent affection, for which reason they must be considered as having originated from the fungus cells, as has been shown to be possible of appearence in cultures, and which seems to have occurred in the seven other rabbits where acid-fast rods had been obtained.

Altogether 9 guinea-pigs and 5 rabbits were inoculated with cultures containing both fungus cells and acid-fast rods, when no tuberculous changes were obtained. The circumstance that acid-fast rods, originating from fungus cells did not produce tuberculous changes, also Reenstierna found, and considered this to be due to the acid-fast rods only being a preliminary stage of tubercle bacilli.

With cultures containing merely acid-fast rods and derived from R 527, passages were made in rabbits, when tuberculoid changes were obtained, which in the second passage, R 689 and 690, were identical with those obtained after the inoculation of ordinary tubercle bacilli. Two rabbits, R 665 and 542, which died after respectively 3 weeks and 16 days, showed in the internal organs chiefly granulomata without necrosis, certainly due to the animals, dying so soon, that typical tuberculous changes had had no time to develop. In the first one of the inoculated rabbits no enlargement of the spleen existed, but in the continued and further passages considerable ones. In all the organic changes were as a rule found plenty of acid-fast rods of the same appearance as Koch's bacilli, lying partly spread about, partly in bundles, some of them being phagocyted,

and also partly in large balls. By subcutaneous inoculation of the acid-fast rods into guinea-pigs abscesses were obtained at the spot of inoculation, which in those animals that lived for a longer time, gradually disappeared. All the abscesses investigated contained acid-fast rods. Only in one guinea-pig was found a lymph gland with an abscess containing acid-fast rods, while the other organs did not manifest any specific changes.

Two rabbits and three guinea-pigs which had been inoculated with acid-fast rods, originating from R 527, were tested with up to 1 mg of a mixture of human and bovine tuberculin, without in any case typical tuberculin reaction being obtained. The same result was obtained in tuberculin tests of rabbits inoculated with the fungus strains.

On cultivating the acid-fast rods from R 527, these grew on Hohn-medium in 14 days in a temperature of 37° C in the form of moist colonies the size of twice a pin's head, while later generations showed unevenly convex colonies with pits in the centre. In culture tests at living-room temperature and temperatures of 45° C, no growth resulted. From a guinea-pig, G-p 383 — inoculated subcutaneously with material containing acid-fast rods from one of the rabbit-passages — cultivation was made from an abscess on the inoculation spot on Löwenstein-medium, when the acid-fast rods, after 14 days at a temperature of 37° C, grew to whitish, very slightly moist colonies of hardly the size of a pin's head, which after another 14 days were somewhat larger.

Summary.

In a historical survey, Chapter I, is rendered a synopsis of the occurrence of yeast-like fungi in healthy human beings and in such with morbid changes, especially in the lungs, and furthermore of the possible relation of these fungi to acid-fast rods, as well as of the mycotic nature of the tubercle bacillus.

Chapter II covers a brief account of the histories of five sanatorium patients who were being treated for pulmonary tuberculosis or were under observation by reason of a suspicion of the same, and from each of which a fungus strain of yeast-type was isolated. In Chapter III is described the establishment of single cell cultures by means of a micromanipulator.

In Chapter IV is rendered an account of some morphological, tinctorial, and biochemical properties of the five fungus strains. Inter alia the capacity of the fungus to produce acid-fast rods is stressed there. Such rods have been found both spread out and lying in major and minor accumulations, and in certain peculiar formations that appeared in five glycerin bouillon cultures from three of the strains.

Chapters V and VI deal with animal tests, with intravenous inoculation of respectively agar and glycerin bouillon cultures of the fungus. The changes in weight of the test animals are illustrated in the majority of cases by diagrams. In Chapter VII is rendered a summary of the animal tests, symptoms and changes caused by inoculation being dealt with; important variations of temperature, often serious loss of weight, disturbances of balance and pareses, and also ocular and other organic changes. The changes of the internal organs were more pronounced shortly after inoculation than later on, with the exception of the eyes, where the changes became manifest late. In one rabbit, R 527, there occurred tuberculoid changes in the lungs, liver, and spleen, and from the two last-mentioned acid-fast rods of Koch's bacillus type were obtained in pure culture. Acid-fast rods were

further encountered once in renal abscesses and twice in ocular abscesses. Furthermore, in four rabbits which nevertheless did not form part and parcel of the series which are being accounted for in detail, acid-fast rods were observed in abscesses.

In the rabbits inoculated with glycerin bouillon culture there were found in the pectoral and abdominal organs fungus cells in altogether fifteen out of fourty-two (likewise also in some inoculated with agar culture), but growth of fungus was obtained only in four out of fifteen. In nine rabbits fungus cells could be proved in ocular abscesses and in three in the central nervous system. In all cases fungus findings were made within 6 months after the last inoculation, with the exception of those in the ocular abscesses and in the central nervous system, where, after the said period, fungus elements were found in seven respectively one of the animals. On cultivation from the nephritic abscesses a growth of fungus was obtained in one case after one day, and a growth of acid-fast rods within seven days.

In Chapter VIII an account is rendered of inoculation into rabbits and guinea-pigs of cultures containing both fungus cells and acid-fast rods, but here no other changes were obtained except such as could be seen after inoculation with only fungus elements.

Chapter IX deals with animal passages with material from the rabbit which displayed tuberculoid changes in the lungs, liver, and spleen. Therewith organic changes were obtained, which in the second passage coincided with tuberculous ones. In the foci there occurred as a rule plenty of acid-fast rods resembling tubercle bacilli, partly spread about, partly in small accumulations, some of them under phagocytosis, and partly in large balls. Inoculation into guinea-pigs never produced generalised tuberculous changes. Only in one guinea-pig, which had been inoculated subcutaneously, was there obtained, apart from an abscess on the spot of inoculation, in a corresponding lymph gland an abscess containing acid-fast rods. Five of the animals inoculated with material containing acid-fast rods, were tested with tuberculin, the result being negative, which was also the case with rabbits inoculated with the fungus strains.

From some of the animals mentioned in Chapter IX cultures were made on Löwenstein medium, when the acid-fast rods grew in a fortnight into yellowish colonies of twice the size of a pin's head, which after recultivation and animal passages displayed a

changed appearance. The acid-fast rods could not be cultivated at living-room temperature or at a temperature of 45° C, nor on any medium unsuitable for tubercle bacilli.

In Chapter X the results of the investigation are discussed and theories for the origin of acid-fast rods from the fungus in question put forward.

Appendix.

Protocols of Inoculated Animals.

R 691.[1] Weight 2,795 gr. Killed 16 days after the inoculation.
Autopsy: Weight 2,725 gr. Small non-raised foci in the kidneys. Otherwise no macroscopic changes of internal organs.
Microscopic examination: The lungs show numerous perivascular infiltrations and endovascular changes in the small vessels. In some places granulomatous, small foci of large, light, plasmatic cells of round shape. The liver does not manifest any changes, and the spleen slight pulp haemosiderosis. In the kidneys are seen a couple of minor, moderately cicatrose striae with round cell infiltrations. The eyes without changes. The brain: In some odd places in the cortex perivascular round cell infiltrations and slight glious proliferation in the surroundings.

R 692. Weight 3,085 gr. Killed 16 days after the inoculation.
Autopsy: Weight 3,050 gr. No macroscopic changes of internal organs.
Microscopic examination: Lungs, liver, kidneys, and ventricle without any changes. In the spleen moderate haemosiderosis and partly in the course of giant cell development. The brain: In several places in the cortex perivascular round cell infiltrations and perivascular granulomata with surrounding glious proliferation. The same condition exists in the central gangliae. In the wall of the third ventricle a compact round cell focus.

R 693. Weight 2,775 gr. Killed 16 days after the inoculation.
Autopsy: Weight 2,910 gr. No macroscopic changes of internal organs.
Microscopic examination: The liver does not show anything pathological, and the spleen trifling haemosiderosis. No changes in the eyes. The brain: In some few places in the cortex round, miliary necrotic granulomata, which contain acid-fast (with Hallberg-stain), tiny bodies, which in size and shape agree with those that have been described by Wright and Craighead (133), and by Levaditi, Nicolau, and Schoen (68) in spontaneous rabbit encephalitis, and by them termed *Encephalitozoon cuniculi.* Minor non-necrotising granolomata in some few places in the subcortical marrow. Perivascular infiltrations here and there in the central gangliae. Slight round cell infiltrations in the soft membranes around the cerebellum.

R 694. Weight 3,115 gr. Killed 16 days after the inoculation.
Autopsy: Weight 3,000 gr. No macroscopic changes of internal organs.
Microscopic examination: Lungs, liver, kidneys, and eyes do not show anything pathological, while the spleen displays moderate haemosiderosis. The brain: In some few places in the cortex miliary and submiliary granulomata, as well as perivascular round cell infiltrations, here and there also in the central gangliae.

R 695. Weight 2,575 gr. Killed 16 days after the inoculation.
Autopsy: Weight 2,590 gr. No macroscopic changes of internal organs.
Microscopic examination: The lungs and liver do not show anything pathological. In one of the kidneys is visible within the cortex and marrow an inflammatory focus

[1] The animals of which an account is given in this appendix occur in the same sequence as in the different chapters. Unless otherwise stated the animals were inoculated with any of the fungus strains, and the rabbits always intravenously.

the size of a hemp-seed, consisting of vast, interstitial round cell infiltrations. Atrophy of the duct system and chronic, productive glomerulitis, besides which numerous collecting ducts are dilated and filled with pus. The brain: Both in the cortex and the cornua ammonis solitary perivascular granulomata without necrosis.

R 697. Weight 3,000 gr. Inoculated with ordinary baker's yeast in maltose agar culture. Killed 16 days later.

Autopsy: Weight 3,160 gr. No macroscopic changes of internal organs.

Microscopic examination: No pathological findings in the liver, kidneys or eyes, while the spleen shows moderate haemosiderosis. The brain: In some places in the cortex small, perivascular granulomata with surrounding glious reaction.

R 698. Weight 3,240 gr. Inoculated like R 697. Killed 16 days after the inoculation.

Autopsy: Weight 3,300 gr. No macroscopic changes of internal organs.

Microscopic examination: Spleen, liver, kidneys, and eyes without pathological changes. Brain: In some solitary spots in the cortex of the cerebrum sparsely located perivascular granulomata.

R 518. Died 3 months after the last inoculation.

Autopsy: Pronounced cachexy. In the upper lobe of the left lung is seen a fibrosed focus the size of a large pea, with a fusion in the centre. Otherwise no macroscopic changes of internal organs.

Microscopic examination: In the lungs venous stasis with diffuse and focal interstitial bleedings with alveolar oedema. In one place, just below the pleural surface, a submiliary necrotic focus with central colliquation. The focus is surrounded by a concentrically stratified capsule of connective tissue and infiltrated with round cells, plasma cells, and leucocytes. In the firm part of the necrosis is seen a large number of round or oval bodies stained with haematoxalin, much larger than lymphocyte foci (suspected fungus cells), furthermore some acid-fast scalds and granules, probably remnants of fungus cells. The spleen shows strong atrophy of the pulp, with considerable coarse-grained haemosiderosis. Fibrous induration of the follicles, and in one or two places miliary epithelioid cellular granulomata without giant cells or necrosis. In the kidneys small fibrous scars, located radially in the cortex. In one of the scars an irregular caseous necrosis without fungus elements or remains of such, and without reaction in the surroundings.

R 519. Died 3 months after the last inoculation.

Autopsy: Cachexy. No macroscopic changes of internal organs. No microscopic examination.

R 520. Died 4 months after the last inoculation.

Autopsy: Cachexy. The spleen smaller than normal. Otherwise no macroscopic changes of internal organs.

Microscopic examination: In the lungs are seen minor areas with atelectasis as well as slight cell accumulations interstitially, and in the liver slight accumulations of round cells in the portal cords. The spleen shows remarkably strong pulp haemosiderosis, with coarse-grained pigment and parenchymatous atrophy, with closely lying trabeculae. In some few places in the kidneys are seen very small round cell infiltrations without necrosis. Suprarenals without remark.

R 521. Died about $3\,^1/_2$ months after the last inoculation.

Autopsy: Cachexy. No macroscopic changes of internal organs.

Microscopic examination: The lungs present here and there slight atelectasis with oedema. In the liver are seen several necroses of submiliary size, clearly fresh, without cell reaction. No micro-organisms can be found. In the spleen moderate pulp haemosiderosis with slight induration of the follicles. The kidneys display some few very thin scars of connective tissue in the cortex, occasionally with trifling round cell infiltrations.

R 522. 3 months after the last inoculation the animal began to hold its head aslant; killed after another 2 months.

Autopsy: No macroscopic changes of internal organs. No microscopic examination.

R 552. 9 weeks after the last inoculation there occurred a serious disturbance of the balance, and after a further 3 months increasing paresis in the hind-legs. Very emaciated. Died about 7 months after the last inoculation.

Autopsy: Cachexy. The lungs without certain changes. The spleen very small, weighing 0.52 gr. On the spleen caulis is seen an almost pea-sized, clear, vesicular formation with a milky-white, excentrically lying nucleus the size of a pin's head. Otherwise no macroscopic changes of internal organs.

Microscopic examination: In one place, beneath the lung surface is seen a partly necrotising focus the size of a hemp-seed, with strong reaction of alveolar epithelium, partly below a formation of giant cells, specially in the periphery of the focus. Some odd fungus cells and fragments of such. No striking similarity to tbc. Otherwise hyperaemia and moderate atelectasis. The spleen shows rather strong pulp haemosiderosis together with collapse of the parenchyma. The kidneys without changes. The central nervous system: Diffused perivascular round cell coatings, and in one place in the cortex cerebri a submiliary tubercle-like granuloma, with central caseous necrosis. In the necrosis are seen plenty of tiny acid-fast organisms, Encephalitozoon cuniculi (cf. R 693).

R 538. Died 9 days after the last inoculation.

Autopsy: The liver pervaded by a large number of white foci as in coccidiosis.

Microscopic examination: In the large biliary ducts of the liver are seen plenty of coccidiae as well as fungus cells of the inoculated type.

R 539. Died 10 days after the last inoculation.

Autopsy: The liver shows on the surface whitish foci strongly reminiscent of those in coccidiosis. Internal organs otherwise without macroscopic changes.

Microscopic examination: In the lungs are seen some atelectatic portions with bleeding. The liver shows a rounded focus of connective tissue rich in cells, with minute necroses, invaded by disintegrating round cells. No micro-organisms could be detected.

R 540. 5 days after the second inoculation there occurred severe disturbance of balance, for which reason the rabbit was not given any more injections. The head was held twisted a quarter of a turn. 3 months later the animal was unable to walk, but could still move its legs, and after a further month it was paralysed in the back part of the body (Pl. XIII, fig. 3), after which it died a few days later.

Autopsy: Very emaciated. Atrophy of the extremities and brittleness of skeleton. Some odd fibrous foci the size of a pin's head in the liver, from which in smears are seen sparse fungus cells and fragments of such.

Microscopic examination: No changes discernible in the lungs, and in the liver only an increase in the periportal connective tissue. The spleen displays a clear, though faint, haemosiderosis. The central nervous system: In several places in the cerebral cortex and in the nuclei degenerative ganglial changes. In one place in the cortex is encountered a small glial cell focus.

R 541. 8 $^1/_2$ months after the last inoculation the rabbit suddenly had convulsions in the extremities and disturbance of balance. The left eye showed rough-waved vertical nystagmus downwards, irrespective of the position of the head, and the right eye displayed similar nystagmus, though upwards. The convulsions grew less after a while, and the nystagmus ceased within 24 hours. In the next few days was noticed an increased oblique position of the head, and the animal was easily brought off its balance, in that it reeled about violently. The disturbance of balance diminished gradually somewhat. The animal was killed 3 $^1/_2$ months after the commencement of the nervous symptoms.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: Lungs, heart, and liver without any changes. The spleen displays moderate pulp haemosiderosis. In one kidney are seen in one spot in the margin of the marrow-cortex miliary granulomata, rich in cells, of uncharacteristic composition. The central nervous system: In the cornua ammonis and at places also in the cortex cerebri, as well as in the subcortical marrow, plenty of perivascular round cell infiltrations with surrounding glial proliferation.

R 553. 8 months after the last inoculation there appeared a permanent, moderate disturbance of balance. The animal was killed $3\,^1/_2$ months later.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: The lungs do not show anything noticeable, and in the spleen is seen a moderate pulp haemosiderosis. In the kidneys very numerous, fine streaks of connective tissue extending through the cortex down into the marrow, and in the liver in a couple of places submiliary granulomata in the parenchyma, with uncharacteristic giant cells. Very little similarity to tbc. Central nervous system: In one place in the cortex cerebri submiliary granulomata without necrosis.

R 554. 2 months after the last inoculation there was noticed hyalitis in both eyes. About a week later manifest loss of strength in the hind-legs, which later on showed distinct atrophy. 2 months after the appearance of the eye-symptoms was noticed a gathering of pus bilaterally in the pupil. The rabbit died $4\,^1/_2$ months after the last inoculation.

Autopsy: Emaciated. The forward parts of the lungs permeated by purulent foci. In smears are encountered some few solitary fungus cells, quite a good many "cones", as well as gram-negative rods. The abdominal organs without macroscopic changes.

Microscopic examination: The lungs display diffuse bronchopneumonia in the early stage of necrotic disintegration (gangraena?). In the liver there exists an uncommonly strong infiltration of round and plasma cells in the periportal connective tissue, but no sure parenchymal foci. The spleen displays hyperaemia and moderate pulp haemosiderosis, and the kidneys some solitary, minute scars in the cortex. Eye: Forward vitreous humour abscess and chronic iridocyclitis with some giant cell formations nearest the abscess. Furthermore, chorioiditic and retinitic changes. No certain fungus cells encountered. Central nervous system: Especially in the lateral parts of the cornua ammonis are seen perivascular round cell infiltrations. In the pedunculus cerebri, on one side, there is found a small infiltration focus of round cells and in one side of the lateral thalamus nucleus a necrosis the size of a poppy-seed, in whose periphery are lying peculiar formations, calcifications? Slight cell reaction in the surrounding areas. No certain fungus cells in the focus. In one place in the marrow of the vermis is seen a focus resembling that in the thalamus, though smaller. In its interior round cell infiltrations.

R 555. About 2 months after the last inoculation increasing paresis of the hind-legs. Died 2 weeks later.

Autopsy: Very emaciated. Contraction of flexors of hip-joints and atrophy in hind-legs. No macroscopic changes of internal organs.

Microscopic examination: Internal organs without changes. Central nervous system: Both in the cortex cerebri and some solitary central gangliae rather small, often perivascular, indistinctly delimited round cell accumulations. The same condition in the marrow of the cerebellum. In the spinal cord are seen not only perivascular round cell infiltrations but also round cell leptomeningitis and remnants of small granulomata, which had clearly been lying between the meninges.

R 556. 2 months after the last inoculation there appeared in the hind-legs moderately pronounced paresis, which was complete after 2 weeks. After another 3 weeks the animal showed dry gangraena in the left paw, which was then infected. Died quite 4 months after the last inoculation.

Autopsy: Cachexy. Infection in the left paw almost healed. The terminal phalanges of the paw protrude bare. Within the lower part of both lungs whitish, confluent

foci, which on pressure being used give off a purulent secretion. A smear shows, apart from sparsely occurring fungus cells, plenty of gram-negative rods, which on cultivation prove to be B. proteus. Spleen small. Abdominal organs otherwise without macroscopic changes.

Microscopic examination: In the lungs purulent bronchitis and diffused purulent bronchopneumonia, mostly of an acute nature, without distinct necrotisation tendency, but in several places with commencing abscess formation. The liver does not show anything noteworthy. The spleen is anaemic, with small follicles, and shows slight pulp haemodiserosis. The kidney displays diffuse, thin streaks of scars through the cortex, with trifling round cell infiltrations. The central nervous system: Light round cell infiltrations in the leptomeninx, especially around the hippocampus. In one pedunele a small round cell and glious focus.

R 557. 5 weeks after the last inoculation there occurred disturbance of balance, and 10 days later moderate paresis in the left hind-leg, and shortly after also in the right one. Decubital lesions in the coccygeal region. The disturbance of balance passed off almost completely very soon. Died almost 3 months after the last inoculation, or approximately one month after the appearance of paresis.

Autopsy: Strongly pronounced cachexy. Superficial granulation around the coccygeal apex, which protrudes bare. Contracture of the flexors of the knee-joints. No macroscopic changes of internal organs.

Microscopic examination: The spleen displays moderate haemosiderosis in the pulp, with giant cell formation and collapse of the parenchyma. No changes in the lungs, liver, and kidneys visible. Central nervous system: Partly in the subcortical marrow, partly laterally around one pedunculus cerebri is found an extremely trifling, sharply delineated, round cell focus. In the leptomeninx of the cerebellum is seen a slight round cell infiltration, while the spinal cord does not present any certain changes.

R 526. Weight 2,100 gr. Killed after 6 months.

Autopsy: Weight 2,150 gr. Lean. No macroscopic changes of internal organs. No microscopic examination.

R 527. Weight 2,100 gr., and 1 month after the last inoculation 1,250 gr. The rabbit was then seriously ill and was expected to die. 2 weeks later there was palpated in the left groin a subcutaneous, rather firm tumour the size of about a butter-pear, and 5 cm. in front of this another such the size of a hazel-nut. After another 3 weeks there became manifest a disturbance of balance. The weight was then 1,375 gr., and the general condition of health was still very low. The disturbance of balance gradually decreased, so that, when the animal was killed 2 months later, 6 months after the first inoculation, only a trifling obliquity of the head was left. The weight was then 1,970 gr.

Autopsy: Emaciated. In the left groin is seen now only one abscess the size of the kernel of a hazel-nut, with detritical content, which in smears proves to contain a moderate quantity of bipolar, gram-negative, non-acid-fast rods. In the right lung, at a height with the hilus, is noticed a white, firm focus fully the size of a pea, with a tendency to umbilication. At a section through this lung, on a level with the said focus, are encountered several small, white nodules. Several such are also present in the left lung. Smears from the right lung show the same gram-negative rods as in the subcutaneous tumour — which on a blood-agar plate grow into non-heamolysing colonies resembling dew-drops — and plenty of *acid-fast rods of Koch's type* (Pl. VIII, fig. 1), as well as solitary *fungus cells* in a state of disintegration. The normal-sized spleen shows on the surface three white foci twice the size of a pin's head, and at a section are noticed plenty of only *acid-fast rods*, which, on cultivation on Hohn-medium without previous sulphuric acid treatment, *grow in pure culture.* The liver is of normal size, with several nodules presenting the same appearance as in the spleen, and contain only *acid-fast rods.* The kidneys are without any macroscopic changes.

Microscopic examination: In the lungs is found a peculiar granulomatous process with granulomata up to the size of a pea. The granuloma cells are epithelioid and often

mixed with uncharacteristic giant cells in large quantities. Here and there in the granulomata focal caseous necroses with remnants of disintegrated cell matter. The *picture reminds one not so little of tuberculosis.* In the liver are distinguished diffuse, very small granulomata, partly with polynuclear giant cells, but without any necrosis (Pl. XVI, fig. 1). In the spleen there occur quite small granulomatous foci of the same appearance as in the liver. Here and there small leucocyte foci; commencing abscess formation? No blood pigment. In the sections from the lungs, liver, and spleen *a fairly large quantity of acid-fast rods* is met with.

R 528. Weight 2,180 gr. After 4 months there occurred cataract in the left eye, and after a further 3 weeks moderate obliquity in the carriage of the head. The rabbit was killed 6 months after the first inoculation.

Autopsy: Weight 2,110 gr., lean. No macroscopic changes of internal organs. No microscopic examination.

R 529. Weight 2,515 gr. Died 9 weeks after the last inoculation.

Autopsy: The liver displays typical coccidiosis. No microscopic examination.

R 530. Weight 2,665 gr. Died slightly more than 3 months after the last inoculation.

Autopsy: Acute rhinitis. Weight 1,385 gr. No macroscopic changes of internal organs. No microscopic examination.

R 558. 6 weeks after the last inoculation there was manifested a weakness in the hind-legs. After a further 4 months there was noticed hyalitis in the left eye, and approximately 3 weeks later also in the right one. The rabbit was then, fully 6 months after the last inoculation, paralysed in the back part of the body. At the same time it contracted rhinitis and died after 4 days.

Autopsy: Brittleness of skeleton; the left fore-leg broke on the animal being stretched on the board for sectioning. On pressure being exerted on the lungs plenty of purulent matter exuded. Smears from the lungs contain small, gram-negative, bipolar rods, which on cultivation grow like pasteurella bacteria. The abdominal organs without macroscopic changes.

Microscopic examination: In the lungs purulent bronchitis without necrosis of the secretion. Pulmonary oedema and slight pneumonic infiltrations. Liver without change. The spleen displays pronounced haemosiderosis. In a section of kidney are seen some quite thin, cicatrose striae in the cortex, and solitary infiltrations. In the left eye there is present an old retinal loosening, in places with proliferation of glious elements and chronic chorioiditis, with solitary, submiliary foci resembling tubercles or changes in sympathetic ophthalmia. Furthermore, there is seen an abscess in corpus vitreum with necrosis and masses of fungus cells. The central nervous system: In some solitary places in the cortex submiliary granulomatous foci without necrosis.

R 559. 2 weeks after the last inoculation commencing paresis in the hind-legs, and a week later the rabbit sat with its hind-legs bent at the hip-joints but otherwise quite straight (Pl. XIII, fig. 1). Died 3 $^1/_2$ months after the last inoculation.

Autopsy: Position of hind-legs as stated above. *Cachectic.* Brittleness of skeleton. No macroscopic changes of internal organs.

Microscopic examination (only the brain examined): In the leptomeninx around the cornua ammonis slight round cell infiltrations. All sorts of degenerative changes in the nerve cells; postmortal changes?

R 560. 2 weeks after the last inoculation there occurred moderate paresis in the hind-legs and 7 weeks later hyalitis in the left eye. After another week was noticed obliquity in the position in which the head was held, and 6 months after the last inoculation hyalitis in the right eye. After another 6 weeks commencing cataract of the left eye. The rabbit had then difficulty in keeping its balance. It was killed 11 $^1/_2$ months after the last inoculation.

Autopsy: On the kidneys are seen minor scars. Otherwise no macroscopic changes of internal organs.

Microscopic examination: Lungs without changes. In some few spots in the liver are seen miliary and submiliary granulomatous foci, composed of epithelioid cells and minor forms of giant cells, and also surrounded by round and plasma cells. *A certain similarity with tubercles* is manifest, though there is no necrosis. The spleen displays severe, coarse-grain haemosiderosis, partly with giant cell formation. In a section of the kidney are noticed sparsely rather narrow and wider radially located cicatrose striae through the cortex, with trifling round cell infiltrations. In one place an interstitial round cell infiltration of miliary size. Both eyes display in the main analogous pictures: Close to the lens, which is partly infected at the edge, is seen a small hyalitic abscess. The central nervous system: In the cortex cerebri, subcortical marrow, and central gangliae small localised granulomata with surrounding glious proliferation. In one cornu ammonis a major granuloma, which fully agrees with the one described for R 693.

R 561. 3 days after the last injection there occurred a severe disturbance of balance. The rabbit was then dying, and was therefore killed.

Autopsy: The lungs show several white nodules, which in smears prove to contain fungus cells. On exerting pressure on the lungs there exudes a purulent secretion. On the liver are noticed some white, not raised foci, which in smears show fungus cells. The organs otherwise without visible changes.

Microscopic examination: The lungs manifest numerous bronchogene abscesses and small purulent bronchopneumoniae. No very great tendency to caseous necrosis within the exudation, a suggestion of same within certain broncho-lumina. Moderate degree of haemosiderosis within the pulp of the spleen. In the liver are seen diffuse necroses. The kidneys manifest a form of chronic nephritis: Innumerable striae with plenty of cells within the cortex, where the glomeruli are atrophic. The straight channels are filled with desquamated epithelium. The central nervous system: In the front part of the cerebrum, far less than in the cerebellum, cortical foci in the form of abscesses with a slight tendency to necrosis. In the environs severe astroglia reaction and perivascular round cell infiltrations. In the cornua ammonis and pedunculus cerebri also in several places similar but smaller foci of decreasing intensity. In some of the foci are found fungus cells in minor units. In the cortex of the cerebellum, too, are seen a number of submiliary abscesses.

R 562 and **563,** died 8 days after the last inoculation.

Autopsy: The kidneys are pervaded by abscesses the size of a pin's head. Otherwise no organic changes visible. In smears from the kidneys plenty of fungus cells. No sections of the organs examined.

R 564. 6 days after the last inoculation there became manifest a serious disturbance of balance, and one month later paresis in the hind-legs, which after a further 3 weeks were paralytic. Slightly more than 2 months after the second inoculation the animal was killed.

Autopsy: Emaciated. In stretching the animal on the board for sectioning the right femur is fractured. In the lungs and kidneys there are noticed small, white nodules, which in smears prove to contain solitary fungus cells. Otherwise the organs are without macroscopic changes.

Microscopic examination: In the lungs are seen major and minor granulomatous foci, whose centres consist of large, pale, plasmatic cells (epithelioid cells), and periferally round cell infiltrations. Nowhere caseous necrosis. Severe hyperaemia and atelectasis. The spleen shows pronounced pulp haemosiderosis. In the liver parenchyma, outside the portal vessels, are seen diffuse, small cell infiltrations and granulomatous foci, some of which latter contain polynuclear giant cells. In the kidneys are seen diffuse, scarified striae through the cortex in connection with round cell infiltrations, as well as here and there fresher granulomatous striae. The central nervous system: In the cortex cerebri and in the hemispherical marrow focal glious infiltrations. In the pedunculus cerebri are seen foci that rather resemble perivascular granulomata, though quite small. Slight pial round cell infiltrations, especially near the cerebellum. In

the basis of pedunculus cerebri none, or trifling, changes. In one of the spinal cord sections are seen in the pyramidal frontal tract slight perivascular round cell infiltrations.

R 565. Fully one month after the last inoculation there occurred the commencement of loss of strength in the hind-legs, and 2 months later the rabbit sat with its legs stiffly extended. After another 10 days that part of the back of the body which is most subject to pressure, presented a purulent, bloody mass, with the left testicle hanging down in its funicle, and therefore the animal was killed.

Autopsy: Right testicle missing, left one as stated above. Ulcerous surfaces in gluteal and perineal regions. No macroscopic changes of internal organs.

Microscopic examination: In the lungs are seen here and there slight parenchymatous blood effusions. The spleen is ostensibly anaemic, with only trifling pigment in the blood in the reticular cells of the pulp. The liver and kidneys show no changes. The central nervous system has not been examined.

R 566. 5 weeks after the last inoculation there was noticed in the hind-legs a loss of strength, which after a few weeks diminished. Approximately at the same time there commenced cataract bilaterally with gradually occurring vascular injection in the sclerae. After 2 months there was present endophthalmia chronica oculi bilateralis. The rabbit was killed 11 $^1/_2$ months after the last inoculation.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: In the lungs there is visible only trifling atelectasis. The liver displays some solitary miliary foci composed of epithelioid cells and round cells, without giant cells or necrotic formations. The spleen manifests severe, coarse-grained haemosiderosis, partly with giant cell formation. In the kidneys fine cicatrose striae through the cortex and a couple of major foci of connective tissue, with slight round cell infiltrations. Both eyes manifest mainly analogous conditions: Endophthalmia chronica with encapsuled hyalitic abscess and glious retinal proliferations. Plenty of lipoid cell accumulations around the abscess, which is seen to contain digested fragments of the lens, which is partly dissolved. The central nervous system: In the meninges, especially around the hippocampus and to a minor degree around the convexity of the brain, round cell infiltrations.

R 567. 5 weeks after the last inoculation there occurred progressive paresis in the hind-legs and the rabbit died after a fortnight.

Autopsy: Emaciated. No macroscopic changes of internal organs.

Microscopic examination: In a section from one of the lungs are seen minor atelectatic portions below the surface, but otherwise nothing remarkable. The liver is without pathological changes. In the kidney are seen generally small cicatrose striae through the cortex, some of them with round cell infiltrations. In one place a granulomatous focus, which is probably lying in the place for a destroyed glomerulus. The central nervous system has not been examined.

R 568. Slightly more than 1 month after the last inoculation there occurred moderate paresis in the hind-legs, which progressed during the next month, but then disappeared altogether. 4 months after the last injection there was seen hyalitis oculi dexter, and 7 $^1/_2$ months later, when the animal was killed, cataract in the same eye.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: The lungs present no changes. In the liver are seen on the border next to the portal connective tissue here and there submiliary granulomata of the same appearance as in R 566. The spleen displays a good deal of blood infusion in the sinus and fairly strong haemosiderosis. In some reticular cells are seen grains and different-sized pellets which are greyish in haemotoxylin-stained preparations and red in Hallberg-stained preparations, these often displaying stronger staining of the perifery. Their nature cannot be closely determined. In the kidneys very sparse, narrow, radially-located striae of connective tissue, partly reaching down through the marrow, with trifling round cell infiltrations in a couple of places. One eye does not

present any changes. The other shows chronic iridocyclitis with old retinal loosening. In a Hallberg-stained section are encountered in a subretinal abscess two small groups of somewhat clumsy, acid-fast rods (in appearance agreeing with those in Pl. VII, fig. 3). The central nervous system: In the cortex cerebri, subcortical marrow, and, roughly outlined, in the cornua ammonis solitary granulomata analogous with those described for R 693.

R 569. 4 weeks after the last inoculation there appeared disturbance of balance and a week later moderate, but afterwards increasing, paresis in the hind-legs. Died $2\,^1/_2$ months after the last inoculation.

Autopsy: Emaciated. No macroscopic changes of internal organs.

Microscopic examination (only the spinal cord examined): No certain changes visible.

R 570. 2 weeks after the last inoculation there occurred moderate paresis in the hind-legs. 14 days later hyalitis oculi bilateralis was noticed. After another month there was present cataract in the right eye and after another 2 weeks also in the left one. The rabbit was killed $11\,^1/_2$ months after the last inoculation.

Autopsy: Emaciated. Apart from minor scars in the kidneys no macroscopic changes of internal organs.

Microscopic examination: In the lungs diffuse minor foci of large cells, partly epithelioid cell composition, reminiscent of small granulomata. No giant cells. No necrosis. The liver displays several diffuse, submiliary granulomata of epithelioid and round cells, as well as partly with giant cell formation. No necrosis. *A certain resemblance to tubercles.* In the spleen is distinguishable moderate haemosiderosis with giant cells. Sections from the kidneys show in the cortex a wedge-shaped, fibrotic focus with retained glomeruli. Trifling round cell infiltration. In one eye is present old retinal loosening of the edge of the lens, with lime incrustations. The other eye shows mainly the same picture, though with slight suppuration. The central nervous system: In the cortex cerebri, subcortical marrow, and cornua ammonis solitary, submiliary, probably perivascular, granulomata. In the spinal cord signs of fresh pyramidal tract degeneration.

R 571. Died 4 days after the second inoculation.

Autopsy: In the left pleural cavity 3 teaspoonful of exudation mixed with blood, but without micro-organisms in smears. The lungs without visible changes. On the left lobe of the liver some whitish, non-raised foci containing some fungus cells. Otherwise no macroscopic changes of internal organs. No microscopic examination.

R 572. 6 weeks after the last inoculation commencing, progressive paresis in the hind-legs. 14 days later hyalitis oculi bilateralis, and after a further 3 weeks cataract in the right eye. Died 4 months after the last inoculation.

Autopsy: Cachectic. No macroscopic changes of internal organs, apart from very minute scars on the kidneys.

Microscopic examination: In the lungs diffuse haemorrhages, otherwise nothing pathological. The spleen displays trifling blood pigment in the reticular cells of the pulp. Liver and kidneys without appreciable changes. Eye (only one eye examined): In the rear lateral part of the bulb a subretinally located abscess encased by the fibrosely altered, hardly recognisable, retina, whose neuroglia is in irregular proliferation. Inside the abscess is seen a fungus colony the size of a millet seed, mainly composed of round and ovoid cells. In the capsule of the abscess is also found a minor abscess in which sparse fungus cells are visible. The central nervous system: Perivascular, slight, diffuse infiltrations in the cornua ammonis and also in the meninges. Trifling round cell infiltrations in the meninges around the cerebellum. Otherwise nothing certain pathological.

R 585. Hardly 2 months after the last inoculation there occurred a slight disturbance of balance and some obliquity in the carriage of the head. The animal was killed $7\,^1/_2$ months after the last inoculation.

Autopsy: The spleen smaller than normal. Otherwise no macroscopic changes of internal organs.

Microscopic examination: Solitary submiliary epithelioid cell granulomata without necrosis in the lungs. Furthermore, purulent bronchitis with aspirated foreign bodies. Heart and liver without changes. In the cortex of the kidney are visible some solitary round cell infiltrations, and in the marrow a fibrose stria. The spleen displays trifling pulp haemosiderosis. The central nervous system: In the cortex cerebri and one side of the central gangliae slight granulomata. The picture is analogous to the one described for R 693. In the basis of the pedunculi cerebri and in the pons arms small, perivascular glial cell proliferations.

R 586. 4 weeks after the last inoculation moderate paresis in the abdominal muscles and hind-legs, and after a further 2 months hyalitis in the left eye, and a few days later also in the right one. 3 months later phtisis bulbi sin. Died 7 $^1/_2$ months after the last inoculation.

Autopsy: Pronounced cachexy. General organic atrophy, especially the spleen remarkably small. In both kidneys plenty of scars. Otherwise no macroscopic changes of internal organs.

Microscopic examination: In the lungs severe hyperaemia and minor haemorrhages. The liver is without any changes. In the spleen exist parenchymatous collapse and considerable anaemia, as well as a trifling quantity of blood pigment in some reticular cells. In sections from the kidney the cortex is seen to be pervaded by a large number of fine cicatrose striae, with a small quantity of round cells, furthermore a large, strongly marked fibrous portion with retained glomeruli and plenty of interstitial round cell infiltrations. The area is delimited from the marrow by a broad, haemorrhagic, hyperaemic zone. In a papillary apex is seen a small focus of round cells and leucocytes. Testes without any changes. Left eye: Endophthalmia chronica with an old abscess, which contains numerous fungus colonies. The right eye presents in principle the same picture, though with somewhat fewer masses of fungus elements. The lens is at the edge seen to be in a state of dissolution, and in the chorioidea layer is seen a large necrotic granuloma. The central nervous system: One-sided, severe hydrocephalus internus. In solitary places in the cortex cerebri minor glial infiltrations, and in the subcortical marrow in several places perivascular infiltrations. In two places close to each other there are existing old malacic foci, one of which contains a small granuloma surrounded by proliferating glial cells. The granuloma cells contain in the centre masses of bluish-green grains (with Nissl-stain), and several necrotic interstratifications, partly of a granular-ropy structure. The second focus contains no granuloma, only some necrotic interstratifications. Very minute glious proliferations in the pons arms. The spinal cord without visible changes in the sections taken.

R 587. 18 days after the first inoculation obliquity of the head set in, and after a further 3 weeks increasing paralysis of the hind-legs, and then of the whole back part of the body. Died 2 $^1/_2$ months after the last inoculation.

Autopsy: Pronounced cachexy. The back part of the body is atrophic. The left kidney shows several scars. On the back of the liver are seen some reddish-white, minor foci. The internal organs otherwise without macroscopic changes.

Microscopic examination: The liver presents some major and minor necroses with plenty of disintegration of nuclei. The foci are located close below the surface and appear to be perfectly fresh, without any surrounding granuloma zone (Pl. X, fig. 1). In several gall ducts are seen necrotic exudations, and in one of the larger ones are found plenty of fungus cells, which in Hallberg-stained sections are partly acid-fast, partly non-acid-fast, and partly not stained at all (Pl. X, fig. 2). In the kidneys the cortex seems to be permeated by numerous cicatrose striae, rather deficient in cells. The central nervous system: In the cerebrum, cerebellum, and pedunculus cerebri nothing certain pathological is noticed.

R 588. Died hardly 8 months after the last inoculation.

Autopsy: Cachexy. On exerting pressure on the lungs there exudes a light, purulent secretion. In the liver are seen several white foci, one of them being the size of nearly a pea. In the kidneys minor scars.

Microscopic examination: In the heart is visible, in foci, light myocardial fibrosis. The lungs display several coccidiosis foci without necrosis. In the liver there are several coccidiosis foci with calcifications. Furthermore, fibrose foci with haemosiderosis. In the spleen there exists strong pulp haemosiderosis, partly with giant cell formations. In the cortex of the kidney and down in the marrow fairly plentiful, fine radially located striae deficient in cells, and also minor fibrose foci. The central nervous system: In some odd places trifling perivascular round cell infiltrations, and in the cortex in one place a small focus composed of very minute granulomata and glial cell proliferations.

R 589. Died 7 days after the inoculation.

Autopsy: The kidneys permeated by white abscesses the size of a pin's head, and in the liver a few such. In smears from the kidney abscesses fairly plenty of fungus cells, as well as some solitary, short, *acid-fast rods*. Otherwise no macroscopic changes of internal organs.

Microscopic examination: The heart displays in some odd spots in the myocardium microscopic granulomatous foci, with diffuse epithelioid cells, without necrosis. In the surroundings necrotic, calcinated muscle filaments. In the lungs are seen some few bronchopneumonic foci of a purulent nature, as well as bronchites and atelectases. The liver manifests strong hyperaemia but otherwise nothing pathological. In the kidneys are seen in both marrow and cortex numerous submiliary abscesses surrounded by more or less distinct granuloma zones. In these granulomata in several places rudimentary, polynuclear formations, possibly representing epithelioid proliferation from duct remnants. No necrosis.

R 590. 5 weeks after the last inoculation increasing paresis of the back part of the body. Died hardly 3 months after the last inoculation.

Autopsy: Pronounced cachexy. Especially the spleen atrophic. Scars in the kidneys Otherwise no macroscopic changes of internal organs.

Microscopic examination: The lungs do not present anything remarkable. In the liver, just near the border to a portal cord, a submiliary necrotic focus with numerous round cells, possibly belonging to the periportal tissue. The spleen displays collapse of the parenchyma and in several places blood pigment in the reticular cells, as well as plasma cells in the pulp. In a section from the intestine nothing pathological is noticed. The suprarenals show in the cortex in one place a slight, fresh necrotic stria. In the kidney diffuse, radiary, narrow striae through the cortex, here and there with sparse round cell infiltrations. The central nervous system: Light round cell infiltrations in the mucous membrane around the hippocampus, and in the region of the fissura sagittalis a preponderating round cell meningitic focus without necrosis. In the foot of pedunculus cerebri a small glious focus.

R 591. 1 month after the last inoculation the rabbit is seen to drag his bent hind-legs. Died 1 week later.

Autopsy: When stretching the animal on the board the left femur fractured just above the knee. Bilateral hydrothorax, in the belly a fairly large amount of a clear exudation, oedema on the back. The heart considerably dilated with coagulated blood in all chambers. In the right chamber are seen on the apices of the papillary muscles and a couple of the cordae tendineae white, verrucose excrescences the size of millet seeds and smaller, furthermore an apparently freelying white, small nodular formation the size of a pea. The trachea considerably vascularly injected, and a moderately mucous coating. In the left lung's lower part a whitish focus nearly the size of a pea, and diffused over the lungs minor such. On the surface of the lung minor haemorrhages. The liver shows an uneven, slightly nodular surface with diffuse, small, whitish foci, which in smears contain a moderate quantities of fungus cells. *On cultivation in glycerin bouillon growth of fungus and after 7 days acid-fast rods.* The other internal organs without macroscopic changes.

Microscopic examination: Sections from the heart substance show solitary subendocardial layers. The excrescences on the papillary muscle apices the size of a millet seed, are composed microscopically of large, cohesive layers of fungus cells, with beau-

tifully radial configuration of the long cells, especially in the peripheries. A hint of concentric stratification. Between the fungus cells are lying masses of disintegrating embedded pus. The surface of the masses of fungus are in free contact with the blood. On the surface of a papillary muscle there is a miliary formation of abscess, surrounded by a thick, close capsule of connective tissue, which in its interior stratum contains some round cells and solitary uncharacteristic giant cells. In the centre of the abscess a small fungus colony with the same appearance as above and with numerous round and ovoid fungus cells. The free formation of the size of a pea is composed of radial fungus masses and detritus mixed with disintegrating pus (Pl. IX, fig. 1). In one place of the formation is seen a cross section of a corda tendinea, in whose necrotic periphery and surroundings are lying large masses of fungi.

Sections from the lung show in one place an older abscess the size of a pea, with necrosis in the centre. In the surroundings slight signs of subchronic pneumonia. The spleen shows very serious hyperaemia with maximally blood-filled sinus. Slightly pronounced haemosiderosis. In several places slight infiltrations of plasma cells. In the liver numerous, irregularly confined necroses without special location, and without manifest cell reaction in the surroundings. Within the necroses the star cells are surviving to a large extent. In some portal vessels fresh thromboses. Fürthermore, plenty of blood pigment in the star cells. In the kidneys radiated and irregular connective tissue scars, chiefly in the cortex. Moderate round cell infiltrations in some of them. The central nervous system: In some few places in the cortex purulently infiltrated, round, submiliary necroses in whose surroundings are seen manifestly perivascular round cell infiltrations. The cerebellum and pedunculus cerebri show nothing certain. In the lumbar part of the spinal cord a glious focus permeated by round cells. In the surroundings hypertrophic astroglia.

R 592. 3 weeks after the last inoculation there became manifest a feebleness in the hind-legs, which 2 months later diminished. Died 4 months after the last inoculation.

Autopsy: On the back a scratch that is healing. The liver shows several whitish foci, which proves to contain pneumococci of type 19 in pure culture. Other organs without visible changes.

Microscopic examination (only the central nervous system examined): In the subcortical marrow some perivascular round cell infiltrations. Indicated granulomatous formation in the cortex in a couple of places.

R 593. Died 4 days after the last inoculation.

Autopsy: Sero-fibrinous pleuritis bilaterally. The lungs pervaded by white nodules the size of a pin's head, as well as larger foci resembling infarcts. The spleen without visible changes. In the liver and kidneys plenty of small, white nodules. In smears from the lungs and the kidneys sparse fungus cells, but on cultivation growth only of gram-negative rods.

Microscopic examination: The lungs pervaded by miliary and submiliary abscesses, which mostly appear to be fresh. In the surroundings of some of the abscesses uncharacteristic giant cells. In major and minor arteries of the lung purulent thromboid masses. The picture agrees with septic emboli in the lung. The spleen shows strong post mortal autolysis, which does not enable a closer judgement. The liver is changed to a high degree in the form of major and minor, partly confluent, fresh necroses, which are mostly localised to centres of the acini. Furthermore, there appear diffuse, quite small, fresh abscesses. In several places thromboses in the finer portal wessels. The kidney displays in the cortex broader and thinner cicatrose striae with sparse round cell infiltrations. Furthermore, small fresh abscesses without any granuloma. In several places dilated tubuli, with disintegrating, purulent content. Solitary fibrose foci in the marrow.

R 594. Died 7 days after the last inoculation.

Autopsy: Bitten in the scrotum so that the left testicle is hanging out of a crustose mass. The lungs show several portions that are lighter and firmer than their surroun-

dings. The spleen is of normal size. Slightly increased delineation in the liver. Smears from the liver contain sparse fungus cells. A couple of haemorrhages with coagulation the size of a 1-shilling coin, in the omentum.

Microscopic examination: In the lungs are noticed here and there perivascular and peribronchial round cell infiltrations. Furthermore, oedema and hyperaemia. The liver displays diffuse, fresh necroses, as a rule in the centre of the acini. Slight infiltration here. The picture agrees with the one in R 593, though it is not so pronounced. In the pulp of the spleen and around the follicles acute, splenitic changes. No large content of blood-pigment. Numerous, fine cicatrose striae, with moderate round cell infiltrations, pervade the kidneys, and also scars with broad wedge-shape in the cortex.

R 595. Hardly 2 months after the fifth inoculation there was noticed cataract in the right eye, and after a further 7 weeks hyalitis oculi sinister. 2 weeks later there appeared obliquity in the carriage of the head. The rabbit was killed $7\,^1/_2$ months after the last inoculation.

Autopsy: Small, whitish, rather firm foci in the right lung.

Microscopic examination: In the lung are noticeable diffuse bronchites, peribronchites, together with bronchopneumonic foci without distinct granulomatous character. The spleen shows trifling haemosiderosis in solitary, swollen, reticular cells. Liver and kidneys without pathological changes. Right eye: Retinal loosening with lipoid deposits in the retinal mass. In one spot in the retinal loosening a largish abscess, in whose centre is lying a large fungus colony of elongated, round, and oval cells. The lens shows within the capsule granular disintegration of the periphery with calcination. Left eye: On the whole the same changes as stated above. The fungus colony, however, shows preponderatingly round and oval cells in the centre and elongated ones in the periphery. In the edge of the fungus colony a peculiar, claviform congestion of the elongated cells. The colony is surrounded by a hyaline border. The central nervous system: In some odd spots in the cortex cerebri and in the vicinity of the third ventricle, glial proliferations.

R 596. 3 months after the last inoculation there was palpated an abscess the size of an almond on the back, in front of a healed scratch. Hyalitis oculi dexter and cataracta oculi sinister. 2 months later some weakening of the hind-legs was noticed. Died 7 months after the last inoculation.

Autopsy: Subcutaneously on the back is located an abscess, sharpley delineated, the size of an almond, which contains only few bacteria of subtilis type. *Pronounced cachexy.* No macroscopic changes of internal organs.

Microscopic examination: Heart, liver and lungs without pathological changes. The spleen shows trifling pulp haemosiderosis. In the kidneys are seen in the marrow-cortex border a diffuse interstitial fibrosis without foci or cell reaction. Right eye: Partial retinal loosening. In one retino-glious proliferation focus with lipoid deposits and cholesterin crystals are seen several small abscesses, partly with necrotic disintegration and here and there also dark, coarse-grained masses (calcinations?). No certain micro-organisms. Left eye: Peripheral disintegration and calcination of the lens within the capsule. Large lipoid granuloma with a central, older abscess between the partially loosened gliously proliferated retina and the chorioidea. In the abscess a large fungus colony, consisting chiefly of elongated cells with claviform swellings in the periphery. The central nervous system: Partial destruction of the pyramidal cell band, with glious proliferation here. No delimited foci. Here and there perivascular round cell infiltrations in the subcortical marrow. Freely lying in the fourth ventricle is seen a lump of exudation the size of hardly a pin's head, the lumen of it being occupied by a fungus colony with a prolific mixture of round, oval, and elongated cells. Indistinct subependymal glial proliferation of the surroundings.

R 597. At the third inoculation some obliquity in the carriage of the head was noticed. The last inoculation was given a week later. After another 5 weeks hyalitis oculi bilateralis, and 2 months later cataracta bilateralis, together with some weakness in the hind-legs. Killed nearly 9 months after the last inoculation.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: No appreciable changes in the lungs, liver, and spleen. The kidneys show some solitary submiliary round cell infiltrations in the cortex and striae of connective tissue lightly infiltrated with round cells. Eye (only one eye examined): Peripheral disintegration with calcination of the lens within the capsule. In the circumference of the rear bulb is noticed a mighty retino-glious proliferation, and in the destroyed retina a largish lipoid granuloma with a large abscess, in whose centre is located a minor fungus colony with preponderatingly long cells. The central nervous system: In a couple of places in the cortex cerebri are found small, fibrosely cicatrose, striate foci with stratified amorphous or coarse-grained masses, which resemble those described for R 586.

R 598. 1 week after the second inoculation there occurred disturbance of balance and gradually progressive paresis, so that the rabbit, 7 weeks after the last inoculation, was paralytic in the hind part of the body, and showed reduced strength in the fore-legs and barrel. Died 1 week later.

Autopsy: Pronounced cachexy. Liver somewhat larger than usual, with a few white, non-raised nodules the size of up to a grain of rice. In the kidneys several white nodules the size of the point of a needle. Other internal organs without macroscopic changes.

Microscopic examination: Lungs without pathologic changes. The spleen shows anaemic parenchyma. Pronounced haemosiderosis in the pulp. In the liver are in some places seen groups of large and small, fresh necroses without distinct reaction in the neighbourhood. The centre of the necroses is often drenched with pus. In smears from these are seen plenty of fungus cells, some acid-fast ones with Hallberg-staining, but the majority unstained, the latter being the case also with Gram-staining. The kidneys display slight, thin, cicatrose striae through the cortex. Immediately below the capsule a small granulomatous focus rich in cells around an inflamatorily changed glomerulus. The central nervous system: In the peduncular gangliae hyperaemia and perivascular round cell infiltrations, and in some few spots small, glious foci. In one cornu ammonis are noticed the same changes, as well as an abscess the size of a poppy-seed, in whose centre are lying vast quantities of unstained fungus cells. The spinal cord without visible changes. In the meninges of the cerebellum and the medulla oblongata round cell infiltrations in a few places.

R 599. 7 days after the second inoculation the animal had grown lean and held its head somewhat aslant, for which reason it was not inoculated then. The last inoculation was made 7 days later. After a further 7 days disturbance of balance. Died after another 11 days.

Autopsy: Pronounced cachexy. A large accumulation of pus is seen to come from the upper part of the mediastinum and then follow the large vessels to the upper thorax apperture, where the masses of pus extend over the left half of the thorax and then descend subcutaneously on the back. Skin intact. No macroscopic changes otherwise of internal organs. The large, totally necrotic, subcutaneous abscess in smears proves to contain non-acid-fast, actinomyces-like elements and gram-negative, slender rods.

Microscopic examination: The spleen displays haemosiderosis. The liver is without visible changes. In the kidneys cicatrose striae in the cortex, which are fairly rich in round cells, and some odd fibrose foci in the marrow. The central nervous system: In the pedunculus cerebri on the one side and in the pons a glial focus. Otherwise hyperaemia in the central gangliae. The spinal cord without visible changes.

R 600. 7 days after the first and only inoculation the rabbit was considerably affected, lean, and after a further 7 days increased disturbances of balance were noticeable. 5 weeks after the inoculation weakness became manifest in the hind-legs, so that the animal after a few more days was unable to support the backpart of its body. 5 weeks later the disturbance of balance still remained, but the paresis had almost disappeared. 4 months after the inoculation hyalitis oculi bilateralis had set in, and after a further month cataracta bilateralis. After that time the rabbit began to increase in weight. It was killed $10\,^1/_2$ months after the inoculation.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: In the heart there is present a focal, slightly myocardic fibrosis. The lungs are without changes. In the liver are seen some submiliary foci but without giant cells. No necrosis. The spleen presents trifling pulp haemosiderosis. In the kidney are visible several slightly round cell infiltrated striae of connective tissue in the cortex and marrow. The right eye presents peripheric necrosis and disintegration of the lens. Retinal loosening with proliferation of glious elements. In the rear of the bulb periphery is visible an older lipoid granuloma rich in connective tissue, with abscess containing a couple of brown fungus cells. Between the pus and the capsule is located a broader zone of epithelioid cell granuloma. The left eye shows the same changes in the lens and total retinal loosening. The central nervous system: Here and there in cornua ammonis are seen minor glious striae and in some of the central gangliae partly perivascular round cell infiltrations, partly a miliary granuloma of the same appearance as the one described for R 693. Subependymally in the fourth ventricle a small accumulation of quite small, perivascular granulomata without necrosis. Furthermore, in the basis of pedunculus cerebri diffuse perivascular round cell infiltrations. Slight round cell infiltration in the mucous membranes around the pons.

R 652. 1 month after the last inoculation there occurred progressing disturbance of balance. 7 months later vascular injection in the sclera of the right eye. Died more than 3 months after the last inoculation.

Autopsy: Cachexy. The spleen is smaller than normal, but the internal organs otherwise without macroscopic changes.

Microscopic examination: In the lungs severe hyperaemia and haemorrhages, and also in one place submiliary atelectatic foci with alveolar proliferation. The liver shows severe hyperaemia and a couple of miliary, fresh necroses. In the cortex of the kidney are seen narrow, cicatrose striae and in the marrow some submiliary abscesses containing large fungus colonies with some odd acid-fast (Hallberg-stain), round and ovoid cells in the centre and elongated cells in the periphery (Pl. VII, figs. 1 and 2). Round the abscesses a fibrose inflammatory capsule (Pl. XI, fig. 1). Right eye: Just behind the zonula cinnii are seen outside the core of the lens three subretinal, miliary abscesses close to each other, one of them necrotically disintegrated. In one of the abscesses is found a fungus colony, which consists chiefly of round and ovoid forms, and in another some solitary fungus cells as well as two small groups of fairly clumsy and short, *acid-fast rods.* Left eye: Retinal loosening with subretinal oedema and some abscesses with large quantities of fungus cells. Just behind the corpus ciliare a quite small collection of pus on the retina. The central nervous system: Within the different parts of the brain diffuse miliary and submiliary malacies in whose surrounding is seen a glial proliferation with peculiarly strong hypertrophic astrocytes. In several places perivascular round cell infiltrations and round cell accumulations in the meninges. The cerebellum displays trifling round cell infiltrations in the meninges. In the spinal cord is found symmetrical degeneration in the pyramidal lateral tracts.

R 653. One month after the last inoculation there appeared moderate paresis in the barrel and hind-legs. Died 5 weeks later.

Autopsy: Slightly reduced flesh. The spleen larger than normal and appearing like an infection-spleen. Smears from the spleen as well as cultivation, though only on Hohn-medium, gave no result. Trifling striae on the kidneys.

Microscopic examination: In the lungs alveolar oedema and hyperaemia. The parenchyma of the spleen seems to be post-mortally changed with severe dissolution of the structure. Furthermore, severe phlegmonoid infiltration and fibrinous precipitation. In some few places submiliary abscesses and severe oedema. The liver, too, was post-mortally changed. In some few places submiliary, purulently permeated necrotic foci. The kidneys display in the cortex numerous narrow, radiary cicatrose striae, which only in a few spots contain round cell infiltrations. In the right eye the periphery of the lens is in globular disintegration, and in the left eye are seen slight round cell infiltrations in the chorioidea. The central nervous system: In the cortex cerebri here and there slight perivascular round cell infiltrations and trifling glious

scars. The same infiltration in the cornua ammonis, central gangliae, and subcortical marrow. In the pyramidal cell band of one cornu ammonis is found an older, miliary, malacic focus permeated by proliferating glia. In the central gangliae, near the third ventricle, a submiliary granulomatous focus with surrounding accumulations of perivascular round cells without necrosis. Plenty of degenerative changes in the centra gangliae (ischaemia).

R 654. Died intercurrently 3 weeks after the last inoculation.

Autopsy: Perforation of the scrotum, so that the left testis is hanging free in its funicle. On the kidneys small scars, while the rest of the internal organs are without any macroscopic changes.

Microscopic examination: Lungs and liver without any pathological changes. In the spleen the pulp is seen here and there to be infiltrated with leucocytes. The kidneys present in the cortex in a couple of places fibrose foci the size of up to a grain of wheat, with trifling round cell infiltration.

R 655. Died intercurrently 3 weeks after the last inoculation.

Autopsy: Macroscopically the same findings as for R 654. No microscopic examination.

R 656. 5 months after the last inoculation there became manifest obliquity in the carriage of the head and some weakness in the musculature of the neck. Killed $2\,^1/_2$ months later.

Autopsy: Apart from a major fibrose focus in the wall of the right ventricle of the heart, no macroscopic changes of internal organs.

Microscopic examination: In the heart is noticed in some spots myocardial fibrosis. The lungs are without pathological changes. In the liver are seen a couple of miliary granulomata, while the spleen shows hyperaemia in the pulp and slight haemosiderosis. Miliary granulomata without giant cells and without necrosis are seen near a papilla in one of the kidneys. The central nervous system: Slight round cell infiltrations in the pia of the brain, especially around the hippocampus. In some odd places in the subcortical marrow perivascular round cell infiltrations. In the basis of pedunculus cerebri and partly also in the pia of the cerebellum, signs of oedema.

R 657. 1 month after the 5th inoculation hyalitis oculi bilateralis, and after another 2 months bilateral cataract. Killed about $7\,^1/_2$ months after the last inoculation.

Autopsy: No macroscopic changes of internal organs.

Microscopic examination: Heart, lungs, and kidneys without any pathological changes. The liver presents some submiliary granulomata (like R 656). In the spleen trifling haemosiderosis. In the right eye retinal loosening, as well as disintegration, and granular calcination of the edge of the lens. In the left eye is noticed, in the rear part of the bulb, within the loosened, gliously altered retina, a lipoid granuloma and in its centre a slight encapsuled abscess, which contains a large fungus colony with hyaline border (Pl. XV). The central nervous system: In the lower central gangliae, in one spot, a submiliary granulomatous focus surrounded by perivascular round cell infiltrations.

G-p 238. Inoculated subcutaneously in the right groin with 1.5 cc of a 35 days old glycerin bouillon culture of strain I containing fungus elements and acid-fast rods. A week later an abscess fully the size of a pea was palpated at the spot of inoculation, but this had vanished after about 2 weeks. 5 months after the inoculation the animal was killed.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 319. Inoculated subcutaneously in the right groin with 1 cc of 62 days old glycerin bouillon culture of strain I containing fungus elements and acid-fast rods. Killed after $2\,^1/_2$ months.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 320. Inoculated like G-p 319, but with 1.5 cc. After a week at the inoculation spot an abscess twice the size of a pea, which 3 weeks later had vanished. Killed 4 months after the inoculation.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 321. Inoculated like G-p 319, but with 2 cc. Killed after 9 months.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 239. Inoculated subcutaneously in the right groin with 1 cc of a 36 days old glycerin bouillon culture of strain III containing fungus elements and acid-fast rods. The animal died after 2 $^1/_2$ months.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-P 333. Inoculated intraperitoneally with 1 cc of a 13 days old glycerin bouillon culture containing fungus elements and sparse acid-fast rods. The culture transferred from Hohn-culture which in its turn had been obtained from a kidney abscess of R 589. Killed after 3 months.

Autopsy: No macroscopic changes of internal organs. No microscopic examination.

G-p 334. Inoculated subcutaneously in the right groin with 1.5 cc of the same culture as G-p 333. Killed after 8 months.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot.

Microscopic examination: The lungs manifest some atelectasis, and the spleen fairly strong haemosiderosis, with some swelling of the sinus walls. The kidneys without changes.

R 687. Weight 1,740 gr. Inoculated with 1 cc of the same culture as G-p 333. After 1 $^1/_2$ month the rabbit showed paralysis in the hind-part of the body. Died slightly less than 2 months after the inoculation.

Autopsy: Weight 1,050 gr. *Strongly pronounced cachexy.* No macroscopic changes of internal organs.

Microscopic examination: The lungs display only some focal haemorrhages and the spleen a slight haemosiderosis. The liver shows nothing pathological. In the kidneys are seen in the cortex solitary, small calcinations of the tubular epithelium. Nothing pathological in the eyes. The central nervous system: In one pedunculus cerebri a loosely built, submiliary focus and in the marrow of the cerebellum, on one side, an older malacic, miliary focus with strong glial proliferation. Solitary, loose glial foci in the pons. (The spinal cord not examined.)

G-p 329. Inoculated subcutaneously in the right groin with 1.5 cc of a 7 days old glycerin bouillon culture containing fungus elements and acid-fast rods (Pl. VI, fig. 1). The culture was made from liver of R 591. After 4 weeks there was observed in the right groin a healed fistula. Killed 8 months after the inoculation.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 330 and **331.** Inoculated intraperitoneally with respectively 1.5 and 1 cc of the same culture as G-p 329. Killed after respectively 8 and 3 months.

Autopsy: No macroscopic changes of internal organs. No microscopic examination.

R 683. Weight 2,660 gr. Inoculated with 1.5 cc of the same culture as G-p 329. After 4 days the rabbit was seriously affected and unable to keep on its hind-legs; died next day.

Autopsy: Weight 2,125 gr. The lungs display on the surface stelliform haemorrhagic foci. The liver is without any changes. The kidneys are pervaded by white nodules the size of a pin's head, which in smears contain plenty of fungus cells and solitary, short, acid-fast rods.

Microscopic examination (only the central nervous system examined): Within different parts of the brain diffuse, miliary abscesses containing large quantities of fungus cells (Pl. VII, fig. 5 and Pl. XII, fig. 3). Moreover, plenty of perivascular infiltrations and meningitic changes. The same picture in the cerebellum and pedunculus cerebri. In the spinal cord, too, are seen diffuse abscesses with fungus cells.

R 684. Weight 2,350 gr. Inoculated with 2 cc 9 days old glycerin bouillon culture made from the liver of R 591 (the same culture as had been inoculated into R 683, though 2 days older). Died after 7 days.

Autopsy: Weight 1,590 gr. *Emaciated.* At the base of the left lung an abscess formation. The spleen fairly small. The liver with no certain changes. No foci. In the kidneys are noticed crowds of generally miliary abscesses in the cortex and marrow. In several of the abscesses are manifested purulent tubuli, and in other scentral necroses, replete with partly unstained fungus cells. Around the necroses radiary location of leucocyte granules. No distinct granulomatous zone of epithelioid cells (Pl. XI, fig. 2).

R 685. Weight 2,680 gr. Inoculated with the same culture as R 684, though twice, with 0.5 cc and 0.75 cc of respectively 11 and 21 days old culture. 1 $^1/_2$ month after the second inoculation the rabbit had lost 850 gr in weight. It then displayed hyalitis oculi bilateralis. The strength of the hind-legs had deteriorated, but this passed off. Gradually increased in weight, and was killed about 9 months after the last inoculation. Bilateral cataract was then noticed.

Autopsy: Weight 2,860 gr. No macroscopic changes of internal organs.

Microscopic examination: Heart, lungs, and liver without changes. The kidneys manifest in the cortex diffuse, thin, fibrose striae and a few cicatrose foci with a trifling quantity of round cells.

R 686. Weight 3,020 gr. Inoculated with 1 cc 11 days old glycerin bouillon culture from the liver of R 591. Died after 18 days.

Autopsy: Weight 1,480 gr. *Emaciated.* Some small, clear, cystiform formations in the mesenterium. Spleen small. Plenty of white, slightly fibrose, non-raised foci in the kidneys. The organs otherwise without macroscopic changes.

Microscopic examination: The lungs and liver do not manifest anything pathological, and the spleen focal, moderate haemosiderosis. The cystiform formations in the mesenterium prove to be thin-walled cysts containing parasites, which certainly belong to the intestinal worms. In the kidneys is seen focal, purulent nephritis with dilated, purulent channels in the marrow and cortex. In some odd spots near the surface of the cortex small abscesses with a few strongly suspected, non-acid-fast fungus cells.

G-p 315. Inoculated subcutaneously in the right groin with an emulsion in NaCl-solution of a crushed part of the lung from R 527. Killed 2 $^1/_2$ months later.

Autopsy: The spleen presents slightly enlarged Malphigian bodies. Otherwise no macroscopic changes of internal organs or at inoculation spot.

Microscopic examination: Lungs and liver without any changes. The spleen shows strong proliferation of the wall-cells in the sinus, which assume an epithelioid type. In this way the sinus is strongly dislocated and filled with blood. The reactionary centres in the Malphigian bodies likewise hyperplastic. No delimited granulomata.

G-p 316. Inoculated like the preceding one, though with a larger dose. 3 weeks later there was palpated in the right groin an abscess the size of a hazel-nut kernel, which had disappeared within 1 month. Killed 10 $^1/_2$ months after the inoculation.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot.

Microscopic examination: Same discoveries as for G-p 315.

R 575. Weight 2,470 gr. Inoculated intravenously with 1.5 cc of an emulsion in NaCl-solution of small bits of spleen from R 527. After 6 months weak in hind-legs. Died 2 weeks later.

Autopsy: Weight 1,310 gr. *Severe cachexy.* Outside the temporal bone a tumour fully the size of an almond, and extending forward towards the orbita, forcing the eye-globe forward, so that a considerable protrusio bulbi sinister exists. The tumour contains a yellowish-white, caseous mass. On both the knee and elbow-joints, as well as in the joint of the left foot, are seen large, nodose, caseous deposits (Pl. XVI, figs. 3 and 4), and towards the columna vertebalis, in the thoracal region, a caseous focus larger than a pea.

In the lungs are seen diffused miliary, whitish foci, and on both apices some abscesses. The spleen is not enlarged and presents on its surface some yellowish-white tuberculosis-like nodules. The liver is without macroscopic changes. The kidneys display on the surface hyaline, cicatrose depressions and in the parenchyma some whitish nodules (Pl. XVI, fig. 2). The ileum and caecum appear to be strewn with sulphur-coloured foci (Pl. XVI, fig. 2).

Microscopic examination: The tumour on the skull seems to be a partly calcinated, caseous abscess and *rather reminiscent of an old tuberculous caseous focus.* In the surrounding capsule are seen *epithelioid cell granulomata with caseous necroses, which resemble to a high degree tubercles* (Pl. XVII, fig. 2). In columna vertebralis and the previously mentioned joints exist caseously necrotic, osteomyelitic foci, surrounded by irregular, broad epithelioid cell borders. Diffuse fresh minute necroses in the bone marrow. Similar foci also in the periosteum. In all the said foci are seen *acid-fast bacilli of Koch's type*, especially in the peripheries of the necroses (Pl. VIII, fig. 5). In the lungs are seen diffuse haemorrhages and numerous microscopic granulomatous foci with epithelioid and round cells. No necrosis. Sparse acid-fast rods. They do not present any very large similarity to tbc. The spleen shows an enormous haemosiderosis and diffuse, large epithelioid cell granulomata with numerous rudimentary giant cells as well as a central necrosis with calcination. Large quantities of *acid-fast rods.* The suprarenals are without any changes. In the kidneys are seen major and minor striae rather deficient in cells, and these striae pass up through the cortex, furthermore fresh cortical granulomata of an elongated form and consisting of masses of epithelioid cells and centrally caseous necroses with *acid-fast bacilli* (Pl. XVII, fig. 1). *Great similarity with tbc.* Granuloma occurs also in the marrow. In the tubuli a couple of more or less purulent secretions. In the center of a necrosis is seen a glomerulus remnant and in this plenty of *acid-fast rods.* Sections from the wall of ileum show in the lymphatic tissue closely lying granulomatous foci. Numerous epithelium cavities are dilated and filled with pus. In one such cavity are encountered a couple of oval, pale yellowish-brown formations with cuticular shell and homogeneously stained content, partly with amorphous, granular masses. (Intestinal parasite?) In the granulomata there are moderate quantities of *acid-fast rods,* partly in small groups. The brain: In solitary spots in the cortex are seen small, glious scars and perivascular, minute granulomata. In the central gangliae of one side an older abscess surrounded by perivascular round cell infiltrations. In the centre of the abscess a necrosis of caseous appearance with a remarkably large number of *acid-fast rods,* partly diffuse, partly in bundles or balls (Pl. VIII, fig. 4). The cerebellum is without visible changes.

R 584. Weight 2,650 gr. Inoculated like R 575, though with 1 cc. Killed after 3 months.

Autopsy: In the central lobe of the right lung a hyaline portion the shape of a clover-leaf. The spleen of normal size, with several white nodules the size of a pin's head on the surface. Otherwise no organic changes visible.

Microscopic examination: In the lung are not seen any granulomatous foci, but here and there epithelioid proliferations in the alveolar walls. No necrosis. The spleen presents diffuse epithelioid cell granulomata with numerous rudimentary giant cell formations, and in some of the foci commencing caseous necrosis. No manifest haemosiderosis. The liver rich in glycogene, but otherwise nothing remarkable.

G-p 307. Inoculated like R 584, though subcutaneously in the right groin. 3 weeks after inoculation there was felt at the spot where the injection was made in the right groin an abscess the size of a hazel-nut kernel.

Autopsy: No macroscopic changes of internal organs. In the right groin an abscess which in smears proves to contain *acid-fast rods.*

Microscopic examination: The spleen presents anaemic pulp and sinus content rich in leucocytes, and also here and there plasmatic cells. No haemosiderosis. Liver and kidneys without changes. The abscess in the groin seems to be encapsuled by a membrane with the appearance of granulomatous tissue rich in cells. In one spot in the same an indistinctly delimited, rounded epithelioid cell granuloma, without giant cells or necrosis.

G-p 324. Subcutaneous inoculation in the right groin with 0.5 mg of 57 days old culture on Hohn-medium of acid-fast rods originating from the spleen of R 527. 10 days later was palpated at the spot of injection an abscess the size of fully a hazel-nut, which after another 3 weeks fistulated. Killed 9 months after the inoculation.

Autopsy: No macroscopic changes of internal organs or at the inoculation spot. No microscopic examination.

G-p 325. Inoculated like G-p 324, though with 0.25 mg. Abscess in the right groin and fistulation as in the above-mentioned animal. Killed $3\,^1/_2$ months after inoculation.

Autopsy: In the right groin are seen two abscesses the size of a pea, and on the right side of the arteria iliaca dexter a lymph gland the size of a hazel-nut kernel which had partly turned into an abscess.

Microscopic examination: In the lungs indicated granulomatous foci without necrosis. The liver without any changes. In the lymph gland is seen an epithelioid cell granuloma with central abscess formation, but without necrosis. Sparse *acid-fast rods.* The lumbar abscess is surrounded by a narrow edge of epithelioid cells. In some spots necrosis in the pus. Rather plenty of *acid-fast rods*, single ones, in masses and under phagocytosis.

R 689. Weight 2,630 gr. Inoculated with 1 cc emulsion of pus from an osteomyelitic focus of R 575 (a speck of pus the size of a pea was emulsioned in 10 cc NaCl-solution). Died $1\,^1/_2$ month later.

Autopsy: Weight 1,610 gr. *Severe cachexy.* The pulmonary parenchyma poorly air-conducting with a few white foci the size of a pin's head. The spleen greatly enlarged (Pl. XVIII, fig. 1), 10 cm long and pervaded by white nodules the size of a pin's head. The liver, too, is enlarged and presents the same foci as in the spleen, though not in such large numbers. On both kidneys diffuse nodules of the same appearance as in the spleen. In the wall of caecum are distinguished a few whitish foci.

Microscopic examination: In the pulmonary parenchyma severely desquamative catarrh and oedema, as well as numerous epithelioid cell granulomata surrounded by lymphocytic casings. In some odd granulomata central caseous necrosis. *Looks like tuberculous changes.* Moderate quantity of *acid-fast rods*, partly diffuse and partly in groups. The spleen is plentifully pervaded by large and partly confluent caseous necroses, surrounded by thinner or broader epithelioid cell borders. Here and there typical Langhans' giant cells. Histologically the changes are *on the whole identical with tuberculous changes* (Pl. XIX). The necroses are pervaded by *enormous quantities of acid-fast bacilli*, especially in the periphery, where they are lying partly diffuse and partly in large balls, and also partly in macrophagi and in the Langhans' giant cells (Pl. VIII, fig. 8). The liver presents *masses of typical miliary tubercles*, the majority with central caseous necrosis and with diffuse Langhans' giant cells. Plenty of *acid-fast bacilli*, diffuse and in groups or balls. In the cortex of the kidney are visible diffuse submiliary granulomata without caseous necrosis or giant cells, partly built up of epithelioid cells. Here and there *acid-fast rods.*

R 690. Weight 2,725 gr. Inoculated like R 689, though with 1.5 cc. Died after 2 months.

Autopsy: Weight 1,300 gr. *Severe cachexy.* The lungs are pervaded by major and minor caseous foci. In the spleen and liver plenty of white nodules hardly the size of a pin's head (Pl. XVIII, fig. 2). In the kidneys a small number of foci. The caecum swollen and pervaded by whitish nodules.

Microscopic examination: As a rule, the lungs display in the parenchyma, especially below the pleural surface, *miliary and major tubercles,* with caseous necroses and purulent admixtures. Diffuse Langhans' giant cells in the surroundings. A moderate quantity of *acid-fast rods.* Histologically judged, the process is of recent origin (Pl. XX). In the spleen are visible severe pulp haemosideroses as well as numerous *miliary tubercles* with central caseous necroses and Langhans' giant cells. Severe reticulo-endothelial hyperplasia in the pulp. Plenty of *acid-fast rods,* singly and in accumulations. The liver presents mainly the same picture as in R 689, but a more pronounced necrotic tendency. Several of the necroses hyalinely encapsuled or metamorphosed, respectively (Pl. XXI, fig. 1). *Acid-fast rods* as in the spleen. In the cortex of the kidney fairly numerous miliary and submiliary granulomata, the larger ones with central caseous necrosis. A moderate quantity of diffuse *acid-fast rods.*

G-p 380. Inoculated like R 689, though intraperitoneally with 1 cc. Weight then 425 gr. Died after about 5 months.

Autopsy: Lean (weight 340 gr.). Internal organs hyperaemic but otherwise without macroscopic changes.

Microscopic examination: The lungs and liver present intensive stasis and the spleen is rich in cells, moderately thickened sinus walls and moderate haemosiderosis. Otherwise no pathological changes.

G-p 381. Inoculated like G-p 380, though subcutaneously in the right groin. Still alive 7 months later.

G-p 383. Weight 425 gr. Inoculated like G-p 381, though with 1.5 cc. 14 days after inoculation there was palpated in the right groin an abscess the size of a hazel-nut kernel, which remained when the animal died within 5 months of the inoculation.

Autopsy: Weight 370 gr. Lean. In the right groin is visible an abscess the size of a hazel-nut, with yellowish-white pus. A slightly enlarged lymph gland on the right arteria iliaca. A slightly increased quantity of moisture in the belly. In the mesenterium two whitish formations the size of a pea. Otherwise nothing pathological visible. The bladder contains a concrement the size of a pea and with a rough surface.

Microscopic examination: Lungs, liver, and kidneys without any changes. In the bladder trifling chronic cystitis. The formations in the mesenterium prove to be lymph glands with lymphatic stasis. The lymph gland near the arteria iliaca presents slight, chronic adenitis, but otherwise nothing pathological. In the right groin a chronic abscess, containing fairly sparse, *acid-fast rods,* and surrounded by an abscess membrane with numerous epithelioid cells.

R 665. Inoculation of 0.2 mg of Hohn-culture derived from an osteomyelitic focus in R 575. Died 3 weeks after the inoculation.

Autopsy: Lungs, liver, and spleen present a fine-grained surface, but no macroscopic foci. The spleen is enlarged.

Microscopic examination: In the lungs numerous epithelioid cell granulomata. No caseous necrosis and no distinct formation of giant cells. Plenty of *acid-fast rods,* diffused and in groups. In the liver lots of quite small epithelioid granulomata, diffusely strewn about and without caseous necrosis. Rudimentary giant cells here and there. No manifest histological similarity to tbc. *Uncommonly plenty of acid-fast rods,* largely in bundles and balls. The paranchyma of the spleen is for the greater part replaced by generally small epithelioid cell granulomata, often arranged as thick casings around the Malphigian bodies. No caseous necrosis and no manifest giant cell formation. *Remarkably plenty of acid-fast rods,* mostly in bundles and balls. The kidney presents acute inflammatory perirenal oedema. In the cortex of one kidney is found

a fibrotic focus about the size of a pea, within which the glomeruli are mostly preserved, but the ducts atrophic. No acid-fast rods.

R 542. Inoculated with 0.2 mg of Hohn-culture from the spleen of R 689. Died after 16 days. No appreciable change in weight.

Autopsy: Spleen enlarged. Otherwise no macroscopic changes of internal organs.

Microscopic examination: The lungs display indications of bronchopneumonic infiltrations and minor atelectatic foci. Moderate quantity of *acid-fast rods*. In the liver plenty of granulomatous foci, which are located pronouncedly periportally, respectively in the periphery of the acini. Some odd minor giant cells but no caseous necrosis (Pl. XXI, fig. 2). Plenty of *acid-fast rods*, largely lying in big heaps and balls. The spleen displays diffuse reticular cell proliferation with sharply delimited epithelioid granulomatous foci, with here and there indicated necroses. Trifling pulp haemosiderosis. *Acid-fast rods* as in the liver. The kidneys show solitary fibrotic cortical striae infiltrated by round cells, partly reaching down into the marrow. In one place in a pyramid a submiliary granulomatous focus of epithelioid cells, surrounded by round cell infiltrations. No necrosis. Some groups of *acid-fast rods*, inter alia in a couple of glomeruli.

G-p 384. Inoculated subcutaneously in the right groin with a bit of the spleen quite the size of a pea from R 690, and emulsioned in NaCl-solution. After about 2 weeks there was palpated a fluctuating abscess almost the size of a walnut in the right groin. 3 months after the inoculation the animal was treated on the back with barium sulphide for the purpose of getting a surface free from hair for tuberculin tests, and the guinea-pig died within 14 hours.

Autopsy: In the right groin an abscess the size of a hazel-nut with large quantities of pus that has penetrated into the femoral muscles. No distinct organic changes.

Microscopic examination: The abscess in the groin is chronic with serious disintegration of the pus. Epithelioid cells of no very distinct character are seen in the surrounding abscess membrane. In the abscess rather plenty of *acid-fast rods*, most plentiful where the disintegration of the cells is greatest. The lymph gland near the arteria iliaca shows numerous submiliary epithelioid cell granulomata without caseous necrosis and without acid-fast rods. The spleen presents strong reticular cell proliferation in the pulp, but no delimited granulomata. Trifling haemosiderosis. In the liver is seen, in conjunction with a portal cord, a trifling epithelioid cell focus.

G-p 385. Inoculated like G-p 384. After two weeks there became visible in the right groin an abscess the size of a hazel-nut, which fistulated a week later. The animal is alive and well 5 months after the inoculation.

R 543. Weight 2,700 gr. Inoculated with 0.1 mg of Löwenstein-culture derived from the liver of R 690. Died 5 weeks after the inoculation.

Autopsy: Weight 2,050 gr. Enlargement of the spleen but otherwise no distinct organic changes.

Microscopic examination: In the lung *numerous miliary and submiliary tubercles. Pronounced histological similarity to tuberculosis.* Moderate quantity of diffuse *acid-fast rods*. In the liver vast quantities of caseous necroses, partly confluent and surrounded by loose, broad zones of epithelioid cells. Only solitary giant cells of Langhans' type. *Great histological similarity to tuberculosis.* Moderate quantity of *acid-fast rods*, here and there plentiful. The spleen presents numerous miliary epithelioid cell granulomata, located especially around the arteries, some of them with central caseous necrosis. Solitary Langhans' giant cells. *Great similarity to tbc.* Findings of *acid-fast rods* as in the liver. The kidneys do not show anything worth noting.

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PLATES I—XXII
WITH EXPLANATIONS

Plate I.

Fig. 1. Microphotograph. Strain III. Four fungus cells after 12 hours' growth at living-room temperature on the surface of maltose agar. The outermost cells show commencing budding. Magnification, 440 diameters.

Figs. 2—6. Show the continued propagation of the fungus cells by budding. Microphotographs taken after respectively 18, 19, 22 $^1/_2$, 26, and 36 hours' growth at living-room temperature. Magnification, 440 diameters.

Figs.

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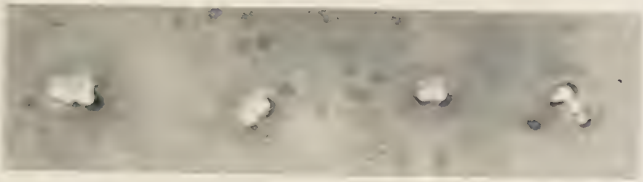

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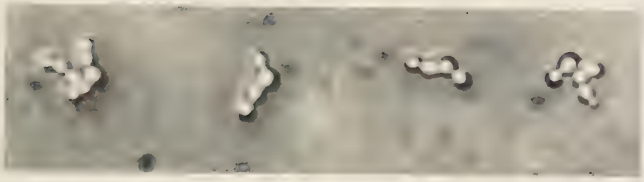

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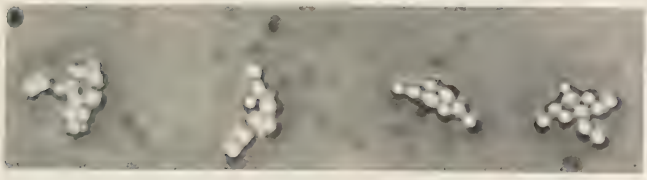

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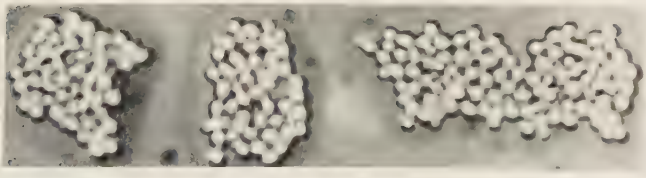


Plate II.

Fig. 1. Microphotograph. Strain II. Part of culture on the surface of ordinary agar plate. Round, ovoid, and elongated fungus cells. Magnification, 660 diameters.

Fig. 2. Microphotograph. Strain V. Part of a culture inside an ordinary agar plate after 24 hours' growth at 37° C. Four full-grown colonies and some groups of fungus cells commencing to form a colony. Magnification, 230 diameters.

Fig. 3. Microphotograph. Strain II. Part of a culture inside an ordinary agar plate after 24 hours' growth at 37° C. In the centre a fungus colony from which issue tendrils of mycelial branches with buds. Furthermore, some more evenly outlined colonies of round and ovoid cells. Magnification, 230 diameters.

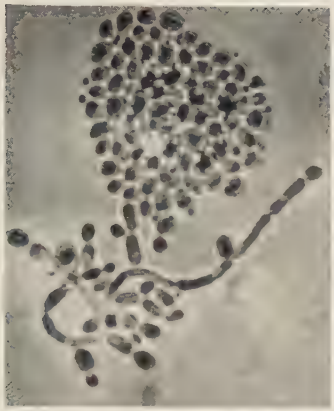


Fig. 1.

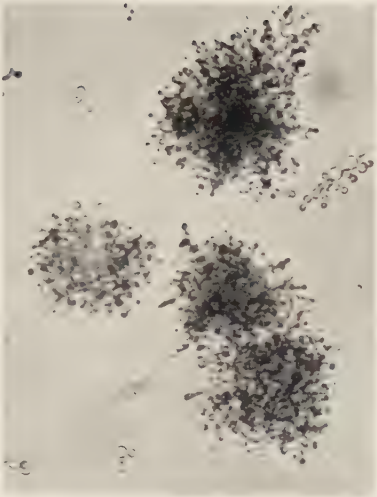


Fig. 2.

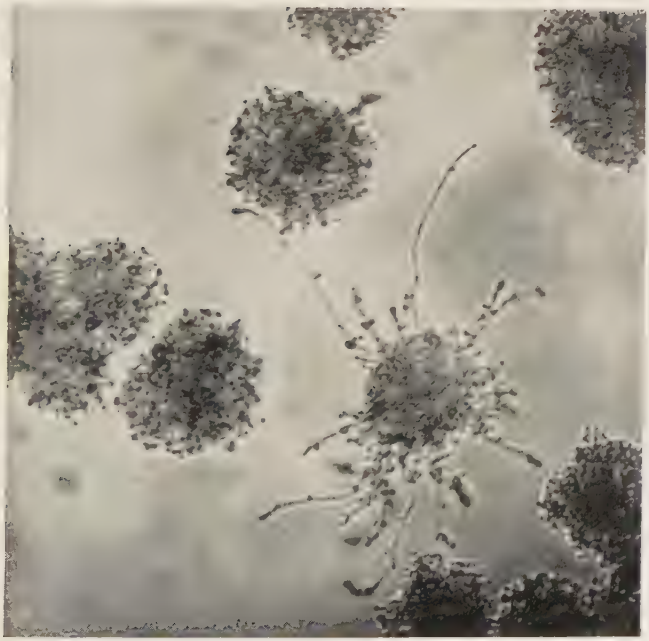


Fig. 3.

Plate III.

(Figs. 1 and 2 are reproduced from a paper (99) by Reenstierna.)

Fig. 1 illustrates a mycotic non-acid-fast micro-organism isolated by Reenstierna in 1912 from the blood of a patient suffering from nodular leprosy in the acute stage. Growing in a special fluid medium it formed, besides solitary, large, prickly cells (one is seen at the top of the figure) chiefly long chains whose links were partly like small yeasts. Fig. 2 shows how some links have changed into acid-fastness. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 3. Strain I. Smear from 15 hours old culture on ordinary agar plate, illustrating the common appearance of fresh fungus cells. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

(Figs. 4—11. Smears from the bottom of one and the same glycerin bouillon culture, originating from *a single cell* of the fungus.)

Fig. 4. Smear from the culture after 36 days at 37° C. In between the fungus cells are seen blue granules with a reddish tint, partly lying in faintly blue-stained disintegrated fungus cells. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 5 and 6. Smear from the culture after 45 days. In fig. 5 are seen, alongside of some fungus cells, non-acid-fast rods, of which a couple with acid-fast granules at one end, and in fig. 6 an agglomeration of non-acid-fast and acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 7—11. Smears from the culture after 36 days. There are seen acid-fast filaments, which are interwoven with the fungus cells (fig. 7), acid-fast, granular rods of the same appearance as Koch's bacilli (fig. 8), as well as amongst the fungus cells acid-fast, dense accumulations of at least at the periphery clearly noticeable rods (figs. 9 and 10). Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 11. Smear from the culture after 45 days. Gram-positive and -negative fungus cells, of which latter one is much larger than the others. In the upper part of the fig. is seen a gram-positive net-work, which with Ziehl-Neelsen stain is acid-fast and appears like that in fig. 7. Stain: Gram. Magnification, 1,000 diameters.

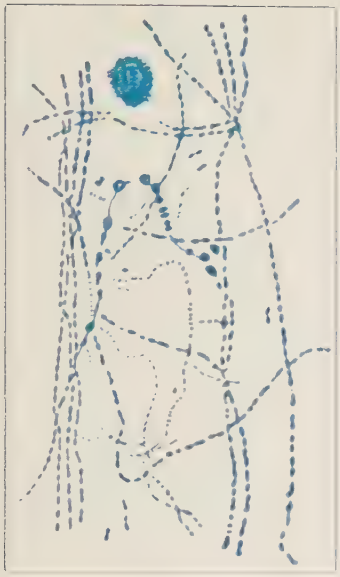


Fig. 1.

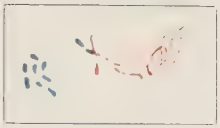


Fig. 2.

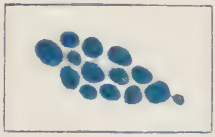
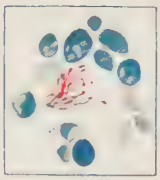


Fig. 3.



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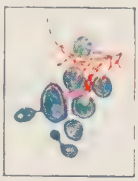


Fig. 7.

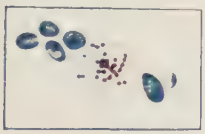


Fig. 4.

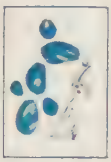
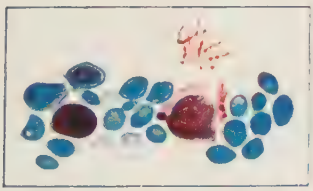


Fig. 5.



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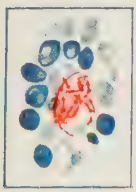


Fig. 9.

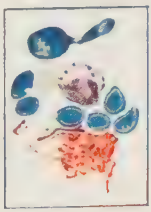


Fig. 10.

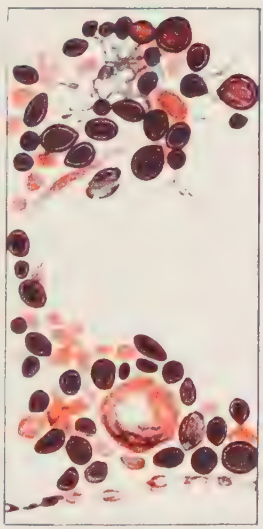


Fig. 11.

Plate IV.

Fig. 1. Smear from the same glycerin bouillon culture as in Pl. III, figs. 4—11, 45 days old. Acid-fast rods and filiform elements, of which one of the latter apparently issues from a fungus cell. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 2. Strain I. Smear from a 62 days old glycerin bouillon culture, which for 30 days has been growing at 37° C and 32 days at living-room temperature. Amongst the acid-fast and non-acid-fast fungus cells, some of which are faintly stained, is seen a vast quantity of granular acid-fast rods. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Figs. 3 and 4. Strain III. Smears from a glycerin bouillon culture after respectively 9 and 13 days' growth at 37° C. In both figures are observed around the acid-fast and non-acid-fast fungus cells amorphous, yellowish-brown masses containing differentiated rods, partly of the same yellowish-brown colour, partly blue (acid-fast). In fig. 3 granular and circular elements are seen forming the outline of light-blue stained, more or less disintegrated fungus cells. In the lower part of fig. 4 are observed some very large, cystiform, brown formations, probably swollen fungus cells in the course of disintegration. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

(Figs. 5 and 6 are reproduced from a paper (41) by Gullberg. They illustrate smears from glycerin bouillon cultures originating from single cells of two of Gullberg's fungus strains of yeast-oidium type.)

Fig. 5. Smear from a 13 days old glycerin bouillon culture of the one fungus strain isolated from the sputum of a patient suffering from pulmonary tuberculosis. There are seen non-acid-fast fungus elements, and in an amorphous mass small granules and small rods, of which a few are acid-fast. The red elements are intermingled in a peculiar manner with the blue components of the amorphous mass. Stain: Fontes. Magnification, 1,000 diameters.

Fig. 6. Smear from a 16 days old glycerin bouillon culture of the other fungus strain isolated from a patient with fever and erythema nodosum in his history. In this figure is seen a large, round formation, whose periphery consists of a wreath of ovoid fungus cells, while its centre is occupied by a net-work of blue filaments, in which latter there are acid-fast rods precisely like the bacilli of Koch. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

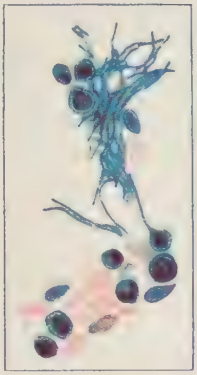


Fig. 1.

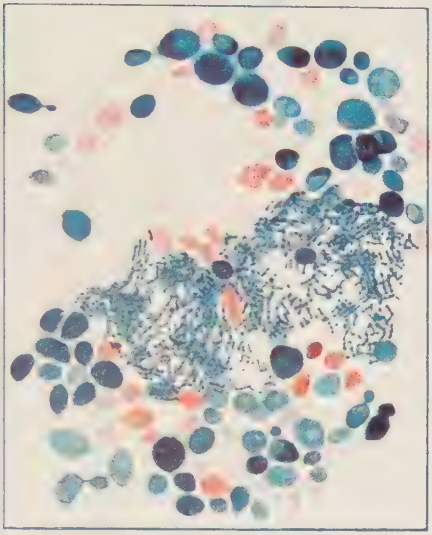


Fig. 2.

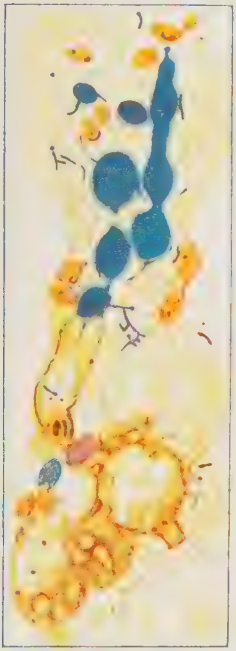


Fig. 4.

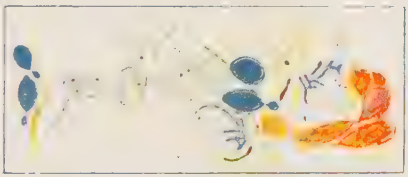


Fig. 3.

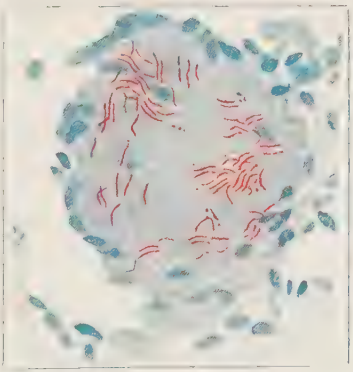


Fig. 6.

Fig. 5.

Fig. 1. Microphotograph. Strain III. Smear from a 42 days old glycerin bouillon culture (with 15 per cent glycerin instead of the ordinary 3 per cent). Amongst the fungus cells are seen diffuse acid-fast rods precisely like the bacilli of Koch. Stain: Hallberg (Nachtblau-pyronin). Magnification, 800 diameters.

Fig. 2. Smear from the same culture as in fig. 1, showing a bundle of acid-fast rods alongside acid-fast and non-acid-fast fungus cells. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 3. Strain I. Smear from a 35 days old glycerin bouillon culture. Blue and red fungus cells and three large, yellowish, peculiar corpuscles covered with red prickles. They agree in appearance with the pine-cone-shaped formations which by Hallberg have been proved in fungus cultures and sputa from patients suffering from pulmonary tuberculosis, and also in the tissues from Schaumann's disease (43). Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

(Figs. 4—12. Smears from a glycerin bouillon culture originating from the liver of a rabbit inoculated with fungus culture. This rabbit, R 591, which was inoculated intravenously five times with glycerin bouillon culture of strain II, died 5 weeks after the last inoculation, when, inter alia, it manifested endocarditis verrucosa mycotica (Pl. IX, fig. 1), as well as necroses in the lungs and liver (Pl. IX, fig. 2). A piece of the liver was transferred into glycerin bouillon, where, after one day, there was obtained growth of fungus, and within 9 days acid-fast rods.)

Figs. 4—6. Smear from the culture after 9 days. In some of the fungus cells are noticed reddish "foci". Fig. 4 illustrates acid-fast rods arranged in a bunch which in size and outline shows similarity with an ovoid fungus cell and lying like a continuation of two such. Figs. 5 and 6 show amongst the fungus cells acid-fast elements arranged in bundles of ovoid form. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 7 and 8. Smear from the culture after 7 days. Fig. 7 shows amongst the fungus cells a formation of intercrossing acid-fast rods. This formation agrees in size and shape with an ovoid fungus cell. In fig. 8 is seen how from a non-acid-fast mycelial branch seems to issue a formation of acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 9. The same smear as in figs. 4—6. Amongst the fungus cells there is a small ball of fairly sparsely lying acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 10 and 11. The same smear as in figs. 7 and 8. Fig. 10 shows amongst long fungus cells, partly in a state of disintegration, a large ball of densely packed acid-fast rods, and fig. 11 two peculiar, acid-fast formations (no doubt fungus elements). Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 12. The same smear as in fig. 9. This fig. illustrates in an agglomeration of ovoid, non-acid-fast fungus cells of ordinary size, a larger acid-fast ovoid cell, ostensibly consisting of closely packed acid-fast elements. At the upper end of this formation there projects a rod-shaped "handle". Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

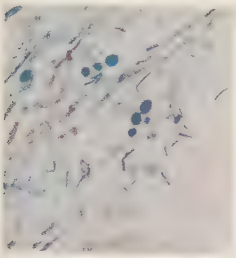
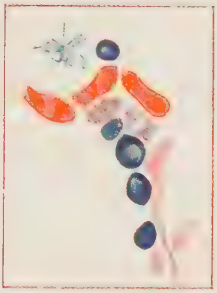


Fig. 1.



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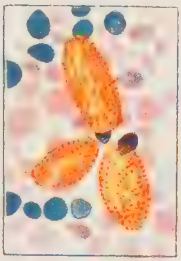

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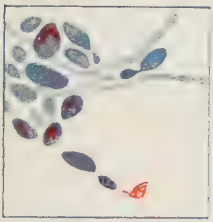


Fig. 4.

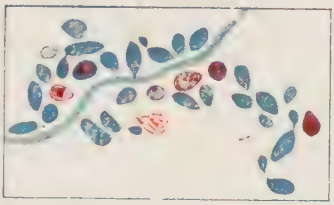


Fig. 5.

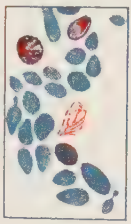


Fig. 6.

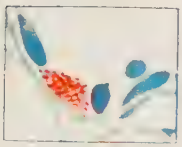
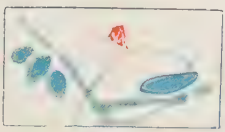


Fig. 7.



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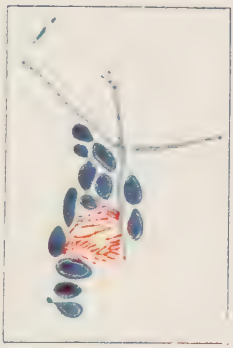


Fig. 9.

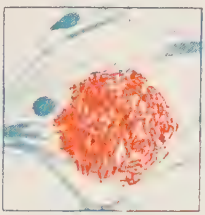


Fig. 10.

Fig. 11.

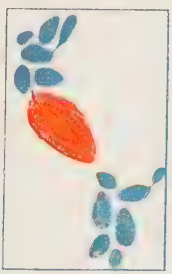


Fig. 12.

Plate VI.

Fig. 1. Smear from the same glycerin bouillon culture as in Pl. V, figs. 4—12, after 7 days. Non-acid-fast fungus elements, particularly mycelial branches, some of which manifest disintegration with surrounding amorphous, granular masses. Three large accumulations of acid-fast rods with the appearance of Koch's bacilli. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 2. Smear from a 53 days old culture on Hohn-medium, originating from a renal abscess from rabbit R 589, which had been inoculated intravenously with 1 cc of glycerin bouillon culture of strain II and died 7 days after inoculation. (Smears from renal abscesses showed fungus cells and very sparse, short acid-fast rods.) This fig. shows acid-fast and almost unstained fungus cells, and also acid-fast granules and short rods. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 3. Smear from renal abscess of a rabbit, which for the preparation of immune serum had begun to be inoculated with a mixture of the five fungus strains, but died 3 days after the second inoculation. An accumulation of granular and rod-shaped, acid-fast elements. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

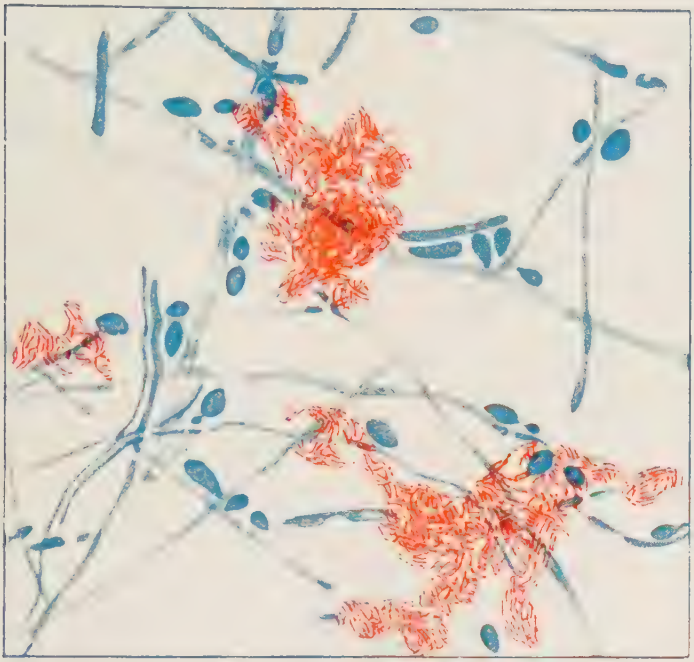


Fig. 1.

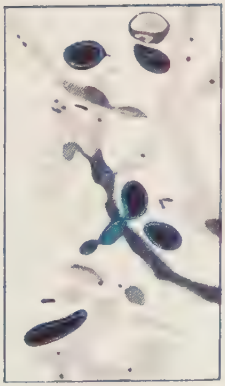


Fig. 2.

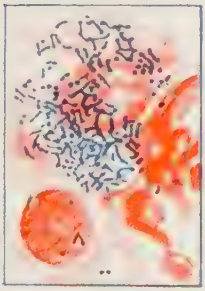


Fig. 3.

Plate VII.

Figs. 1 and 2. Microphotographs of smears from a renal abscess (Pl. XI, fig. 1) of a rabbit, R 652, which had been inoculated five times with glycerin bouillon culture of fungus strain IV, and died hardly $3\,^1/_2$ months after the last inoculation. Fig. 1 illustrates fungus cells of which several have not been stainable at all or only very slightly so. Stain: Gram. Magnification, 1,000 diameters. Fig. 2 shows solitary acid-fast fungus cells, while the bulk are unstained. Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

Fig. 3. Part of section of a subretinal abscess in the eye of the above-mentioned rabbit. A bunch of fairly clumsy and somewhat short, acid-fast rods, is seen. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 4. Part of a section (Pl. XII, fig. 2) from the brain of a rabbit, R 561, which had been inoculated intravenously three times with glycerin bouillon culture of strain I and died 3 days after the last inoculation. In an abscess is seen a chain of non-acid-fast, double-contoured fungus cells. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 5. Part of section from the brain (Pl. XII, fig. 3) of a rabbit, R 683, which had been inoculated intravenously with a 7 days old glycerin bouillon culture containing fungus elements and acid-fast rods. This culture had been made from the liver of a rabbit, R 591 (Pl. V, figs. 4—12 and Pl. VI, fig. 1). In an abscess is seen an agglomeration of slightly violet-stained fungus cells, some of which are plentifully provided with granules, which impart to the cells a certain likeness with the pine-cone-forms described by Hallberg. Stain: Nissl. Magnification, 1,000 diameters.

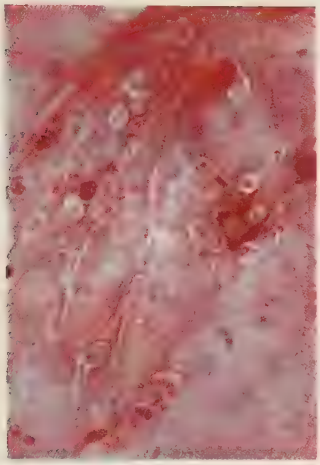


Fig. 1.

Fig. 2.

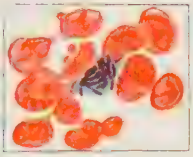


Fig. 3

Fig. 4.

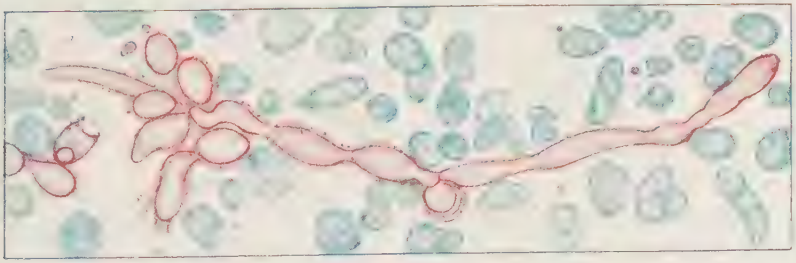


Fig. 5.

Plate VIII.

Fig. 1. Microphotograph. Smear from the right lung of a rabbit, R 527, inoculated intravenously twice with glycerin bouillon culture of strain II. The animal was killed after about 6 months. The autopsy showed macroscopically tuberculous changes in the lungs, liver, and spleen. Histologically, there were observed in the lungs epithelioid cell granulomata with caseous necroses. The picture reminded one not so little of tuberculosis. This fig. shows acid-fast rods of the same appearance as typical Koch's bacilli. Stain: Hallberg (Nachtblau-carbol-fuchsin). Magnification, 1,000 diameters.

Figs. 2 and 3. Smear from the spleen of R 527. Histologically, the spleen showed granulomatous foci, some of them with polynuclear giant cells but without necrosis. Foci of the same appearance were also found in the liver (Pl. XVI, fig. 1). The figs. illustrate groups of acid-fast rods. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Fig. 4. From a section of the brain of a rabbit, R 575, inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. R 575 died in an extremely cachectic condition $6\,^1/_2$ months after the inoculation, and displayed changes in lungs, liver, spleen, kidneys, intestine, and skeleton, some of them reminiscent of tuberculosis (Pl. XVI, fig. 2—4, Pl. XVII). This fig. shows a small portion of a cerebral abscess with partly diffuse bundles of acidfast elements, arranged similar to globi the same as leprosy bacilli, and partly a largish ball, composed of minor balls or tufts of acid-fast elements, so that the whole has a fan-like appearance, Stain: Hallberg (Nachtblau-neutral-red). Magnification, 1,000 diameters.

Fig. 5. Smear from an osteomyelitic focus of R 575. Acid-fast rods, partly in a bundle, and partly some diffuse ones. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

Figs. 6 and 7. Smear from an abscess in the right groin of a guinea-pig, G-p 325, inoculated subcutaneously in the right groin with 0.25 cc of a 57 days old Hohn-culture, originating from the spleen of R 527. G-p 325 was killed $3\,^1/_2$ months after the inoculation. Fig. 6 shows diffuse, acid-fast rods and fig. 7 a phagocyted group of such. The phagocyte cell is in a state of dissolution. Stain: Hallberg (Nachtblau-pyronin). Magnification, 1,000 diameters.

Fig. 8. Part of a section from the spleen of a rabbit, R 689, inoculated with pus from an osteomyelitic focus of R 575. R 689 died after $1\,^1/_2$ month severely cachectic and with organic changes as in tuberculosis (Pl. XVIII, fig. 1, and Pl. XIX). This figure illustrates acid-fast rods, mostly in large, fan-shaped agglomerations. Stain: Ziehl-Neelsen. Magnification, 1,000 diameters.

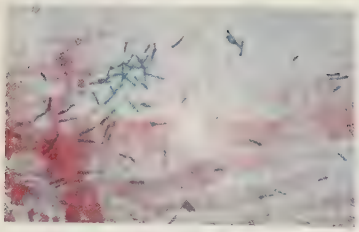
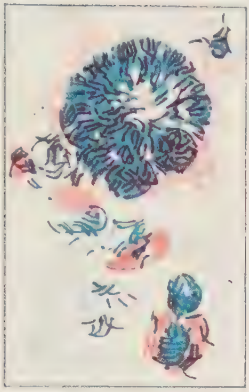


Fig. 1.



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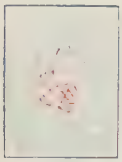

Fig. 2.

Fig. 3.

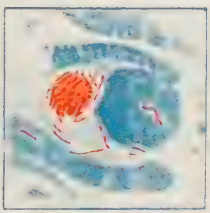

Fig. 5.

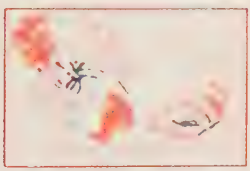

Fig. 6.

Fig. 7.

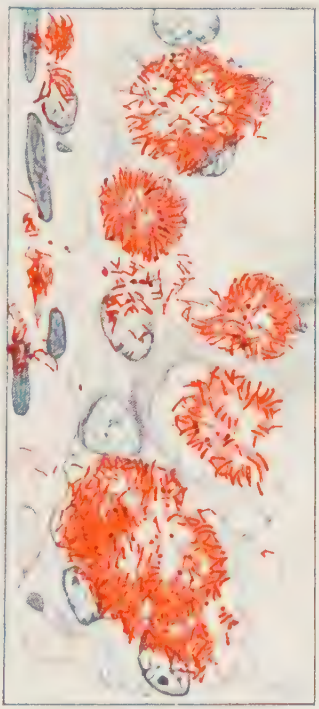

Fig. 8.

Plate IX.

Fig. 1. R 591. (See explanation of Pl. V, figs. 4—12.) The border portion of a mycotic focus the size of a pea on a chorda tendinea in the right cardiac chamber. Stain: Hallberg (Nachtblau-neutral-red; decoloration with propylic alcohol). Microphotograph. Magnification, 340 diameters.

Fig. 2. R 591. Two mycotic-necrotic foci in the liver. (A piece of the liver was transferred into glycerin bouillon, where, after the lapse of one day, fungus elements, and after 9 days, acid-fast rods, appeared (Pl. V, figs. 4—12, Pl. VI, fig. 1). Stain: Haem. v. Gieson. Microphotograph. Magnification, 29 diameters.

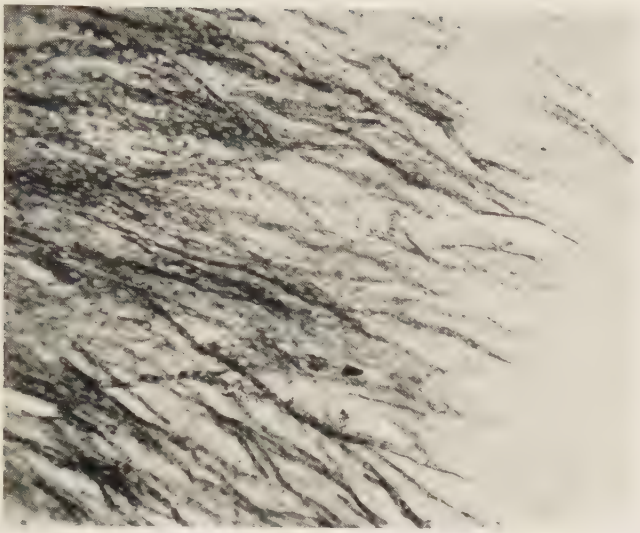

Fig. 1.

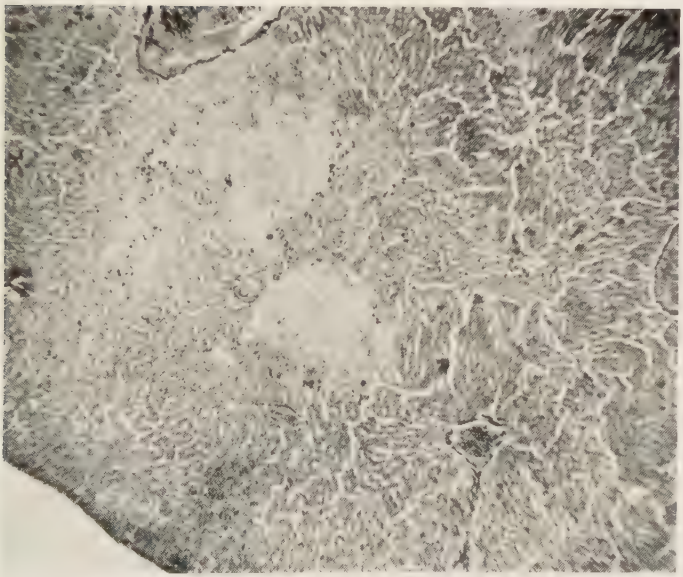

Fig. 2.

Plate X.

Figs. 1 and 2. R 587. Inoculated intravenously five times with glycerin bouillon culture of fungus strain I, died $2\,^1/_2$ months after the last inoculation with pronounced cachexy. Fig. 1 shows necrotic abscesses in the liver. Stain: Haem. v. Gieson. Microphotograph. Magnification, 95 diameters. Fig. 2 illustrates a larger hepatic gall-duct containing fungus cells, some of which are unstained. Stain: Hallberg (Nachtblau-neutral-red). Microphotograph. Magnification, 1,000 diameters.

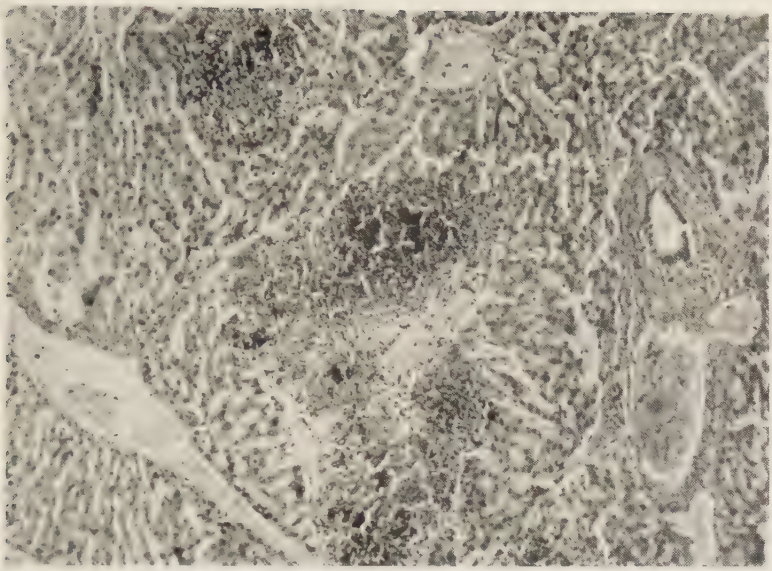

Fig. 1.

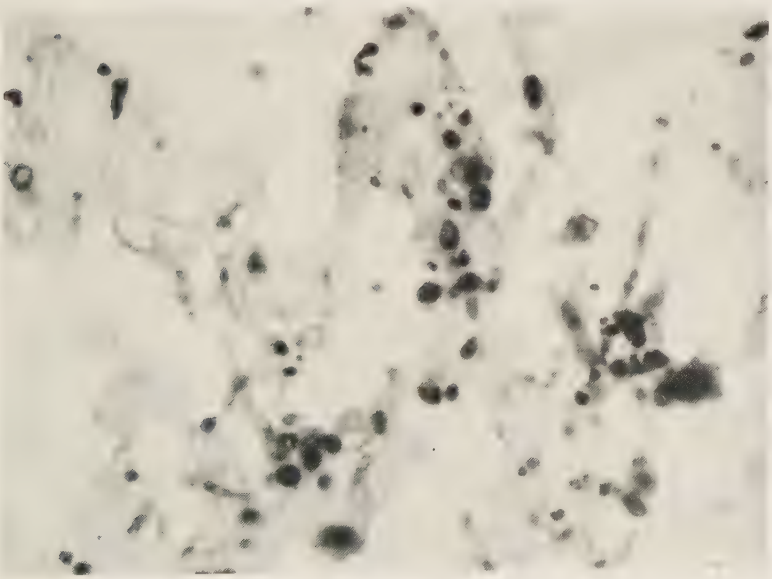

Fig. 2.

Plate XI.

Fig. 1. R 652. Inoculated intravenously five times with glycerin bouillon culture of strain IV, died cachectic 3 months after the last inoculation. This fig. shows in the marrow of the kidney two older abscesses with disintegrating pus. (In the pus plenty of fungus cells, as Pl. VII, figs. 1 and 2, show.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 75 diameters.

Fig. 2. R 684. Inoculated intravenously with glycerin bouillon culture containing fungus elements and acid-fast rods. This culture had been made from the liver of R 591. R 684 died after 8 days. This fig. illustrates miliary, fresh abscesses in the border between the medulla and cortex of a kidney. (Analogous pictures have been obtained also after inoculation with merely fungus cells.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 40 diameters.

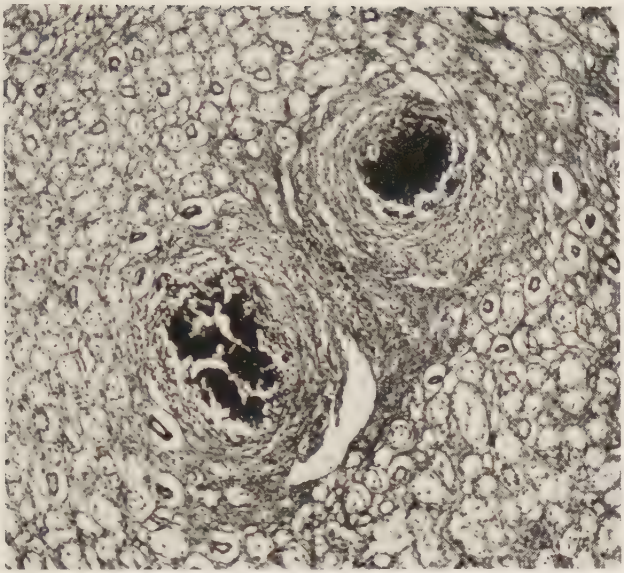

Fig. 1.

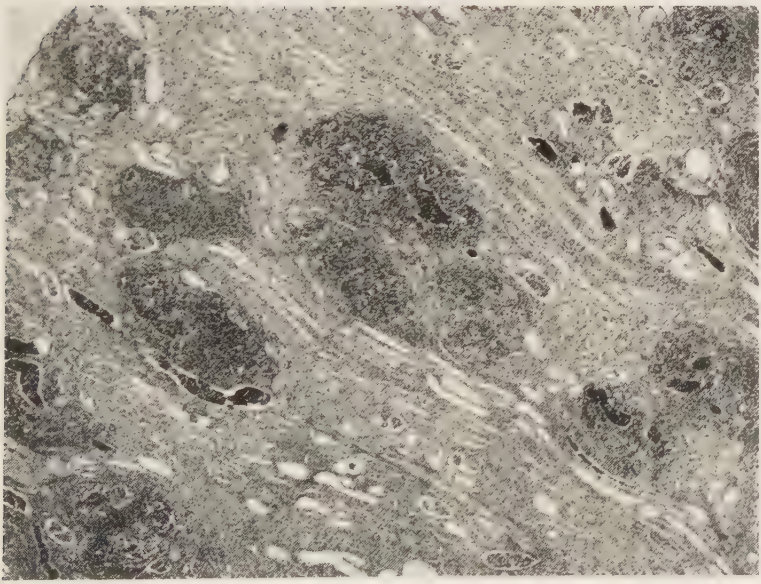

Fig. 2.

Plate XII.

Fig. 1. R 587. (See explanation of Pl. X.) Total view of cross section of a kidney. The dark, radiary striae correspond to connective tissue cicatrices deficient in cells. Stain: Haem. v. Gieson. Microphotograph with low magnification.

Fig. 2. R 561. Inoculated intravenously three times with glycerin bouillon culture of fungus strain I, died 3 days after the last inoculation. Total view of frontal section of the brain, where multiple miliary abscesses are observed, especially in the cortex. (Fungus cells in one of these abscesses are illustrated in Pl. VII, fig. 4.) Stain: Nissl. Microphotograph with low magnification.

Fig. 3. R 683. Inoculated intravenously with glycerin bouillon culture containing fungus elements and acid-fast rods. This culture had been made from the liver of R 591. R 683 died after 5 days. This fig. shows in the central region of the brain a conglomeration of mycotic abscesses. (Fungus cells from here are illustrated in Pl. VII, fig. 5.) Stain: Nissl. Microphotograph. Magnification, 75 diameters.

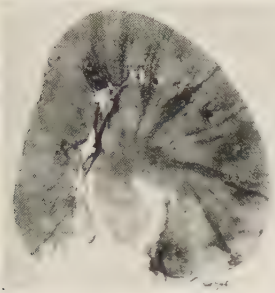

Fig. 1.

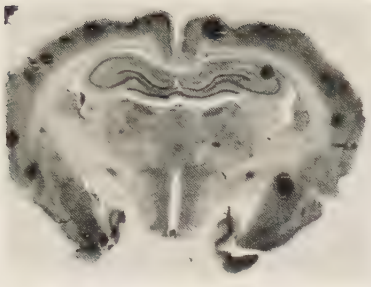

Fig. 2.

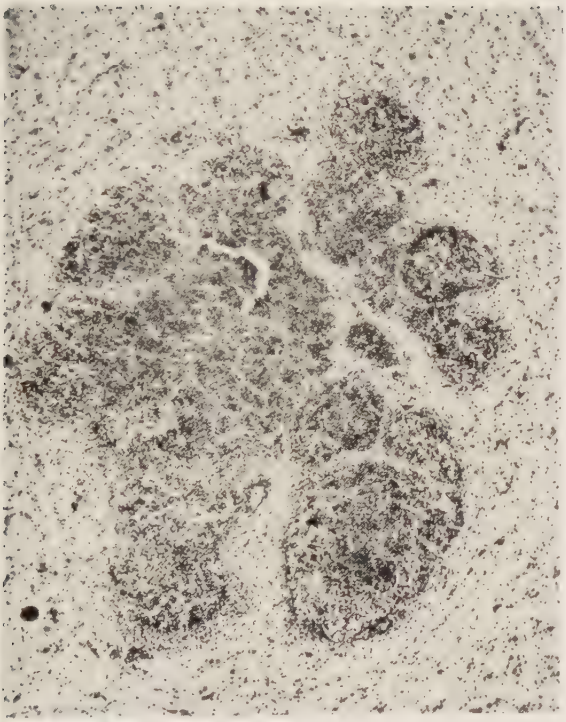

Fig. 3.

Plate XIII.

Fig. 1. R 559. Inoculated intravenously three times with glycerin bouillon culture of fungus strain I. Two weeks after the last inoculation there appeared progressive paresis in the hind-legs, and after another week commencing contracture of the flexors of the hip-joints as well as in the extensors of the knee and foot-joints. Photograph taken 3 weeks after the commencement of the contracture.

Fig. 2. R 569. Inoculated intravenously three times with glycerin bouillon culture of fungus strain IV. Four weeks after the last inoculation there became manifest a progressive disturbance of balance and one week later also progressive paresis in the hind-legs. Photograph taken 14 days after the commencement of the disturbance of balance.

Fig. 3. R 540. Inoculated intravenously twice with slanted agar culture of fungus strain IV. Nine days after the last inoculation there became manifest a severe disturbance of balance and after 3 months paresis in the hind-legs. One month later there was present paralysis of the hind-part of the body. Photograph taken $2^1/_2$ months after the beginning of the disturbance of balance.

Fig. 4. R 564. Inoculated intravenously twice with glycerin bouillon culture of fungus strain III. Six days after the last inoculation there appeared a severe disturbance of balance and one month later paresis in the hind-part of the body, which after some time became paralytic. Photograph taken 7 weeks after the beginning of the disturbance of balance.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Plate XIV.

Figs. 1 and 2. R 596. Inoculated intravenously five times with glycerin bouillon culture of fungus strain III. Three months after the last inoculation there was observed hyalitis oculi dexter (fig. 1) and cataracta oculi sinister (fig. 2). Died 7 months after the last inoculation with pronounced cachexy. (Microscopically, there were present in the right eye great retinal changes, with abscesses, but without any certain fungus cells being found, and in the left eye also pronounced retinal changes with a large subretinal abscess, containing a large agglomerated fungus colony, as well as peripheral disintegration of the lens.)

Fig. 3. R 657. Inoculated intravenously four times with glycerin bouillon culture of fungus strain III. Five weeks after the last inoculation was observed hyalitis oculi bilateralis, and 2 months later cataracta oculi bilateralis. This photograph shows subtotal cataract in the right eye. (Regarding microscopic pictures, see Pl. XV.)

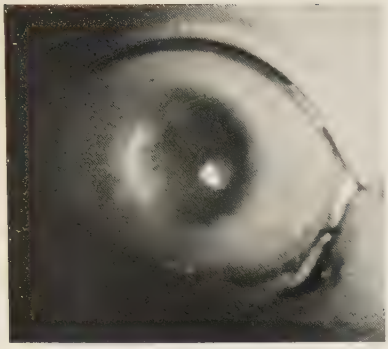

Fig. 1.

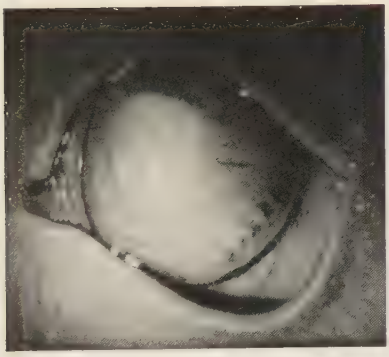

Fig. 2.

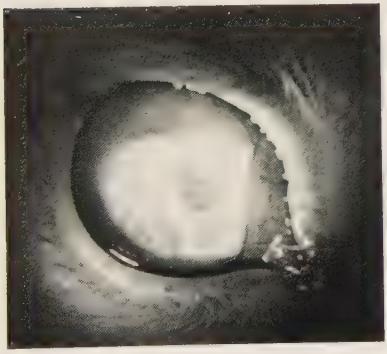

Fig. 3.

Plate XV.

Figs. 1 and 2. R 657. (See Pl. XIV, fig. 3.) Fig. 1 shows an intraretinal, chronic abscess with central fungal focus. Stain: Haem. v. Gieson. Microphotograph. Magnification, 31 diameters. Fig. 2 shows a detail picture of a border portion of the fungal focus. The latter, which consists chiefly of elongated cells, is surrounded by a hyaline border (a). Stain: Haem. v. Gieson. Microphotograph. Magnification, 280 diameters.

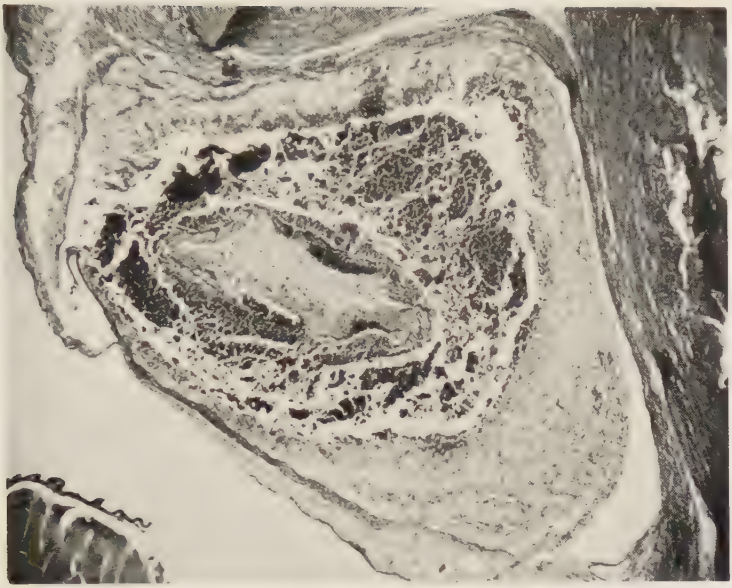

Fig. 1.

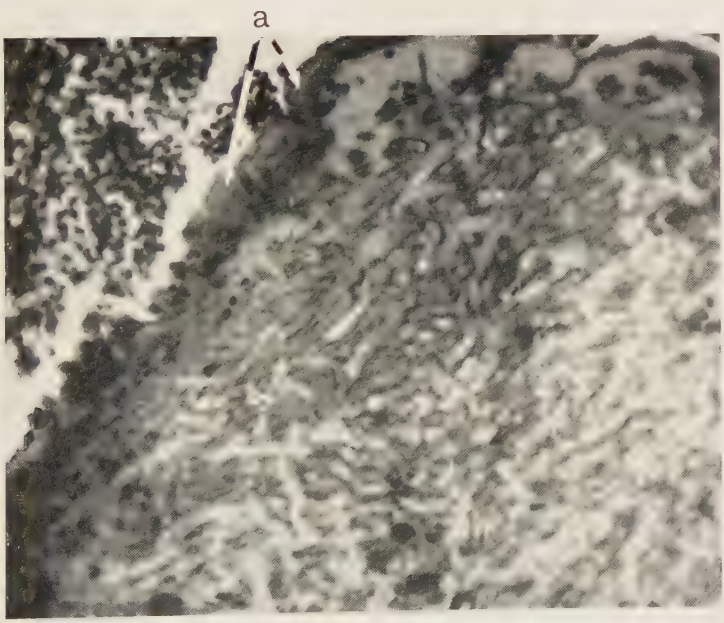

Fig. 2.

Plate XVI.

Fig. 1. R 527. Inoculated intravenously twice with glycerin bouillon culture of fungus strain II, and killed after about 6 months. This fig. shows a tuberculoid granuloma in the liver. At the upper border of the granuloma is seen a Langhans' giant cell. (In the lungs and spleen were found the same changes with a moderate quantity of acid-fast rods of Koch's type, as shown in Pl. VIII, figs. 1—3.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 200 diameters.

Fig. 2. R 575. Inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. The animal died after $6\,^1/_2$ months in an extremely cachectic condition, and displayed changes with acid-fast rods in lungs, liver, spleen, kidneys, intestine, and skeleton, some of them reminiscent of tuberculosis. This photograph shows in the caecum (a) and ileum light foci scarcely the size of a pin's head, and at the top of the photograph a part of the left kidney with cicatrose pits on the surface.

Figs. 3 and 4. These photographs show respectively in the regions left elbow and left knee-joint large, nodose, caseous abscesses, issuing from osteomyelitic foci. (Acid-fast rods from a smear from the focus in the left knee are seen in Pl. VIII, fig. 5.)

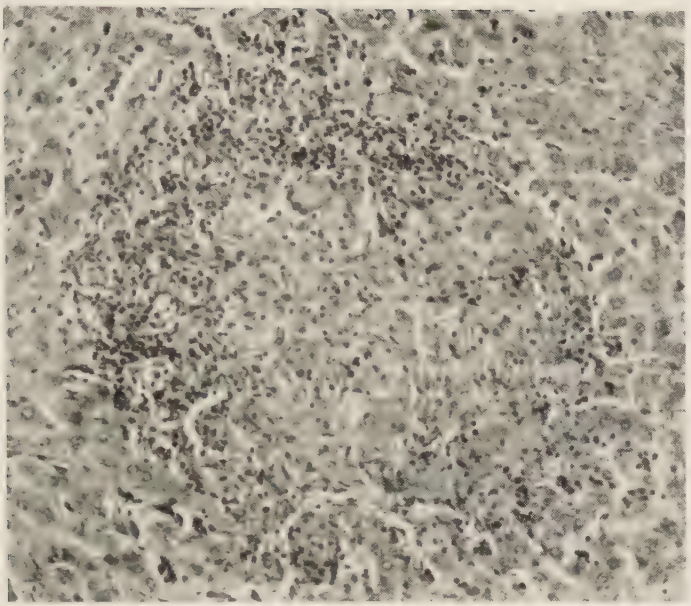

Fig. 1.

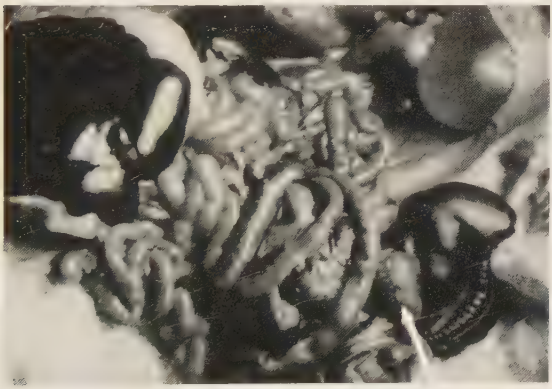

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Fig. 2.

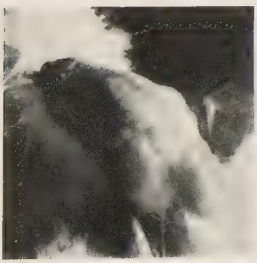

Fig. 3.

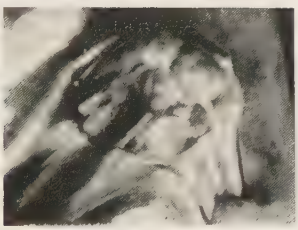

Fig. 4.

Plate XVII.

Fig. 1. R 575. Miliary, tuberculoid granuloma in the cortex of a kidney. Stain: Haem. v. Gieson. Microphotograph. Magnification, 75 diameters.

Fig. 2. R 575. Tubercle-like granulomata (a) with central caseous necrosis in the fibrous capsule of a larger, partly calcified, caseous abscess (b) in the fossa temporalis.

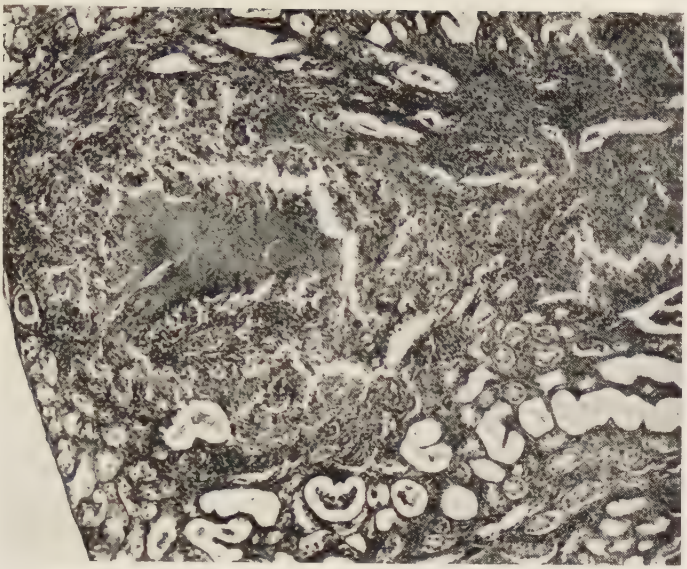

Fig. 1.

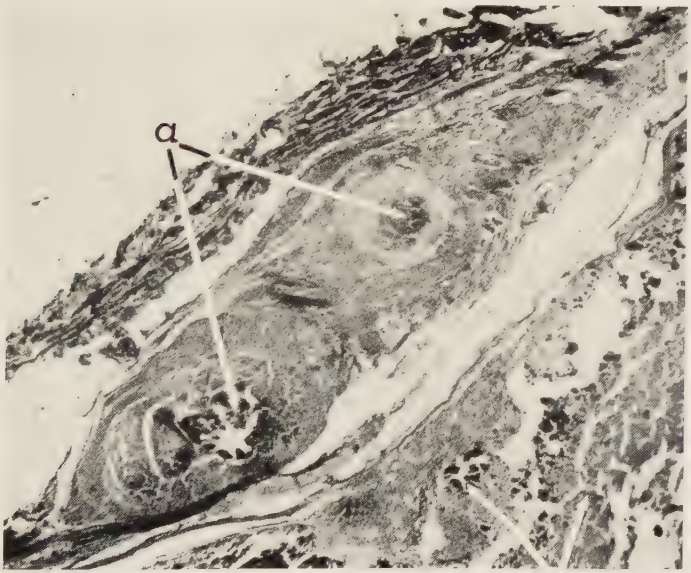

Fig. 2.

Plate XVIII.

Fig. 1 a. R 584. Inoculated intravenously with a suspension in NaCl-solution from the spleen of R 527. The animal was killed after 3 months. This fig. shows the spleen with solitary, whitish nodules glinting through the surface. (Microscopically the nodules were found to consist of epithelioid cell granulomata with numerous rudimentary giant cells, and in one and another granuloma commencing to turn into a caseous necrosis with rather plenty of acid-fast rods.) Photograph slightly reduced. At the autopsy the spleen measured 48 × 13 mm.

Fig. 1 b. R 689. Inoculated intravenously with a suspension in NaCl-solution of pus from the osteomyelitic focus in the left knee-joint of R 575. This fig. shows the excessively enlarged spleen pervaded by numerous miliary foci. (Pl. XIX shows the histological picture.) Photograph in natural size.

Fig. 2. R 690. Inoculated with the same emulsion as R 689. On the left side of the picture is seen a part of the liver with numerous submiliary, white foci. On the right of the picture is observed a bit of one lung with numerous white foci the size of up to a pea. Photograph in natural size. (Microscopic pictures in Pl. XX and Pl. XXI, fig. 1.)

Fig. 1.

Fig. 2.

Plate XIX.

Figs. 1 and 2. R 689. Fig. 1 shows a total view of a part of the spleen with numerous more or less confluent, caseous tuberculoid foci. Stain: Haem. v. Gieson. Microphotograph. Magnification, 6 diameters. Fig. 2 is a detail view of the preceding. (In the spleen plenty of acid-fast bacilli, as shown in Pl. VIII, fig. 8.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 90 diameters.

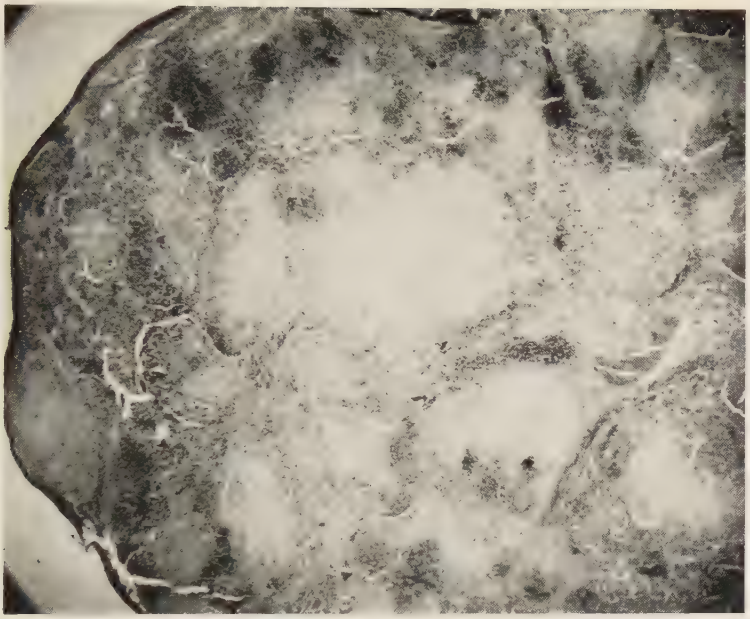

Fig. 1.

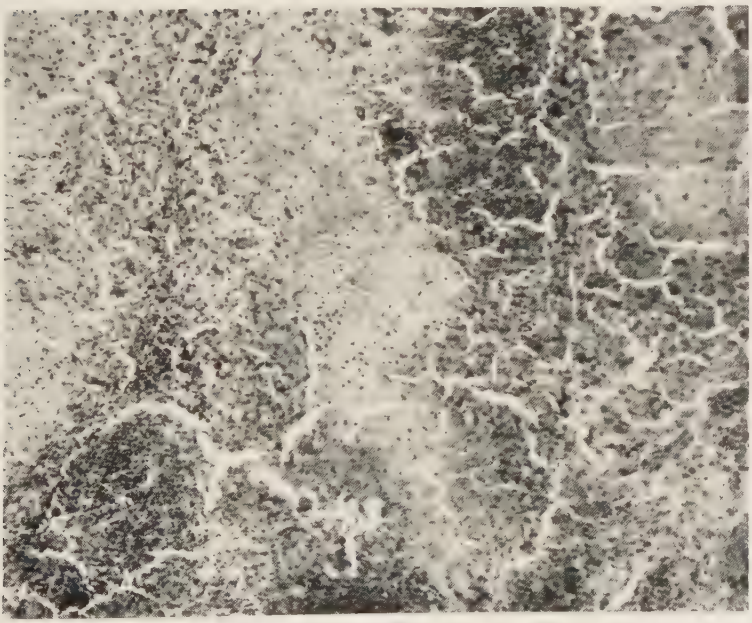

Fig. 2.

Plate XX.

Figs. 1 and 2. R 690. Fig. 1 shows a total view of a part of the lung (Pl. XVIII, fig. 2) with three miliary, caseous, tuberculoid foci, one of which is located subpleurally. The central focus is seen to have developed from the wall of an artery (a). Stain: Haem. v. Gieson. Microphotograph. Magnification, 20 diameters. Fig. 2. Detail view from border portion of one of the said foci in fig. 1. a) caseous necrosis, b) epithelioid cell granulomatous tissue, c) Langhans' giant cell. (In the foci a moderate quantity of acid-fast rods.) Microphotograph. Magnification, 180 diameters.

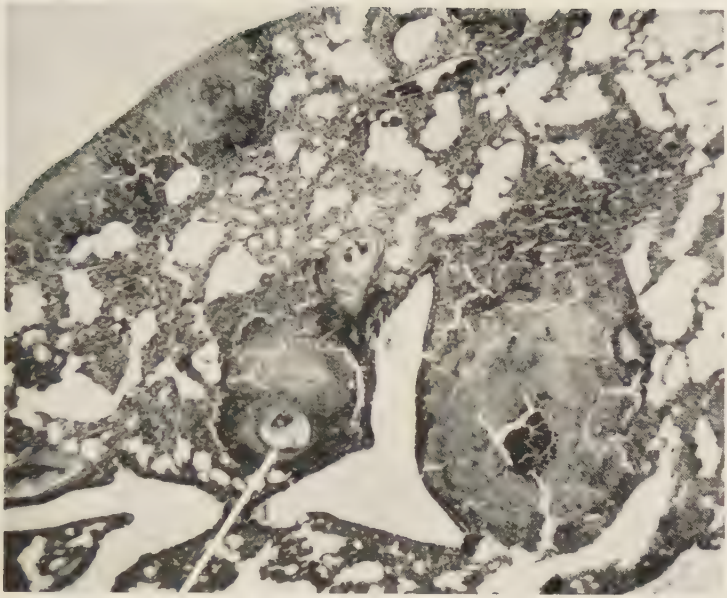

Fig. 1.

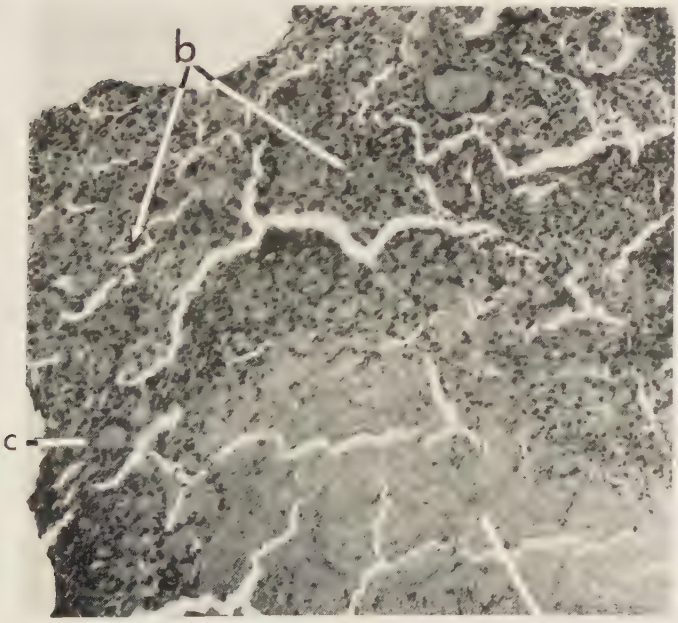

Fig. 2.

Plate XXI.

Fig. 1. R 690. A group of submiliary tuberculoid foci in the liver, with central caseous necrosis (Pl. XVIII, fig. 2). a) a Langhans' giant cell. (The foci contain plenty of acid-fast rods.) Stain: Haem. v. Gieson. Microphotograph. Magnification, 29 diameters.

Fig. 2. R 542. Inoculated with 0.2 mg of a Löwenstein-culture of acid-fast rods from the spleen of R 689. The animal died after 16 days. This fig. shows in the liver an agglomeration of submiliary, fresh, epithelioid cell granulomata pervading the acini but without caseous necrosis. Stain: Haem. v. Gieson. Microphotograph. Magnification, 104 diameters.

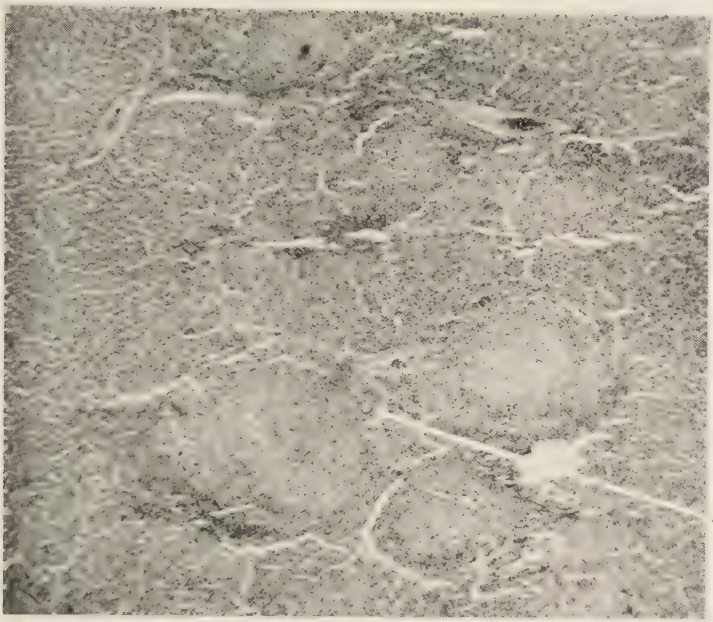

Fig. 1.

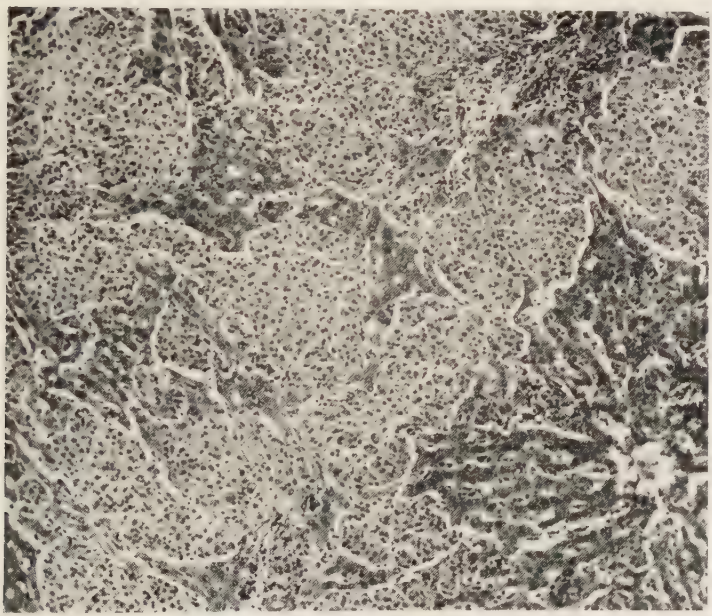

Fig. 2.

Plate XXII.

(Figs. 1—9. Photographs of cultures in natural size.)

Figs. 1—3. These figs. show the growth of fungus strain II on respectively ordinary agar (fig. 1) and maltose agar (figs. 2 and 3) after 10 days, 5 days at a temperature of 37° C and 5 days in living-room temperature.

Fig. 4. Culture on Löwenstein-medium after 53 days at 37° C of fungus strain III, after animal passage. (This culture contained fungus elements and short, acid-fast rods, as Pl. VI, fig. 2, shows.)

Fig. 5. Culture of acid-fast bacilli from the spleen of R 527 (Pl. VIII, figs. 2 and 3) on Löwenstein-medium after 30 days at 37° C, third generation.

Fig. 6. Culture of acid-fast bacilli from the spleen of R 584 (Pl. XVIII, fig. 1) on Löwenstein-medium after 30 days at 37° C.

Fig. 7. Culture of acid-fast bacilli from an osteomyelitic focus in the left knee-joint of R 575 (Pl. VIII, fig. 5, and Pl. XVI, fig. 4) on Löwenstein-medium after 30 days at 37° C, third generation.

Fig. 8. Culture of acid-fast bacilli from the spleen of R 689 (Pl. VIII, fig. 8, Pl. XVIII, fig. 1, and Pl. XIX) on Löwenstein-medium after 30 days at 37° C.

Fig. 9. Culture of acid-fast bacilli from a subcutaneous abscess of G-p 383 on Löwenstein-medium after 26 days at 37° C. This animal was inoculated subcutaneously in the right groin with a suspension in NaCl-solution of pus from an osteomyelitic focus of R 575.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.